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(54) **SET OF PROBES FOR THE DETECTION AND TYPING OF 46 HUMAN PAPILLOMAVIRUS MUCOSAL TYPES**

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C07H 21/04 (2006.01)
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2561/113; C12Q 2523/109; C12Q 2525/155;
C12Q 2527/107; C12Q 2563/179

See application file for complete search history.

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(57) **ABSTRACT**

We have developed a set of probes to detect and identify 46 types of mucosal human papillomaviruses (HPV). These probes recognize the variable region comprised between the 2 conserved regions of the published GP5+/GP6+ set of PCR primers. The 46 probes have been shown to hybridize, as intended, to the DNA derived from the following HPV types: 6, 11, 13, 16, 18, 26, 30, 31, 32, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 67, 68, 69, 70, 71, 72, 73, 74, 81, 82, 83, 84, 85, 86, 87, 89, 90, 91 and 97. The hybridization of each probe is specific for each type without any cross hybridization among types and it is sensitive enough to allow detection of PCR products for genotyping of HPV DNA contained in clinical samples.

6 Claims, 5 Drawing Sheets

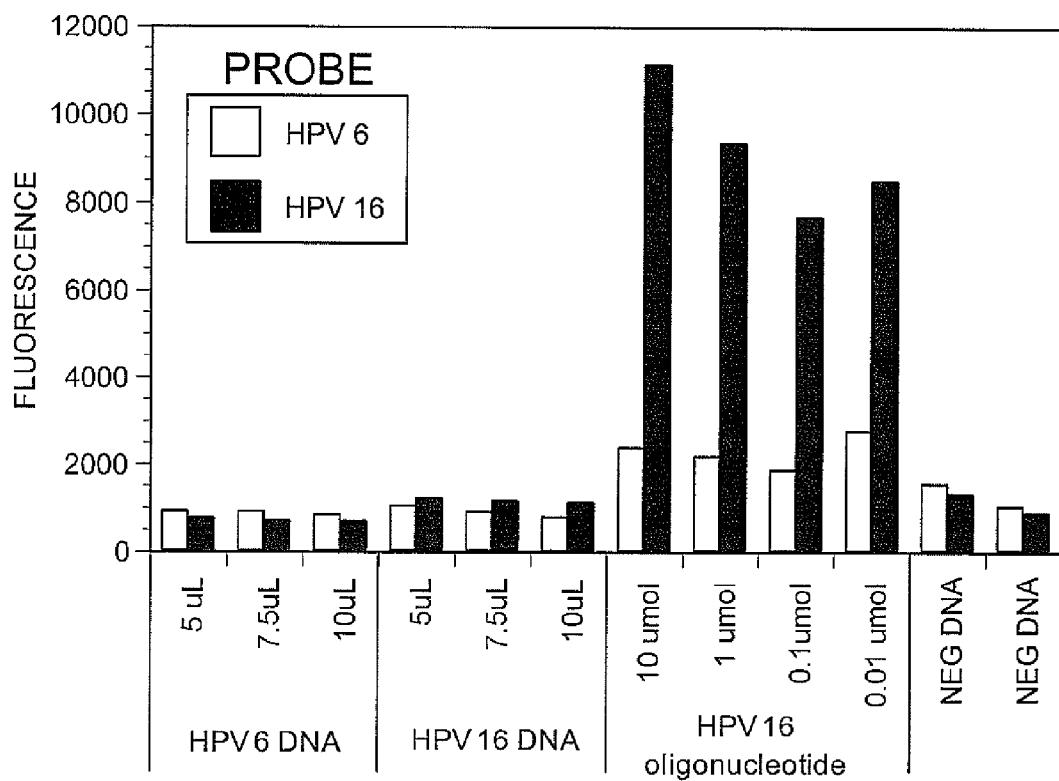


Figure 1.

Fig. 2

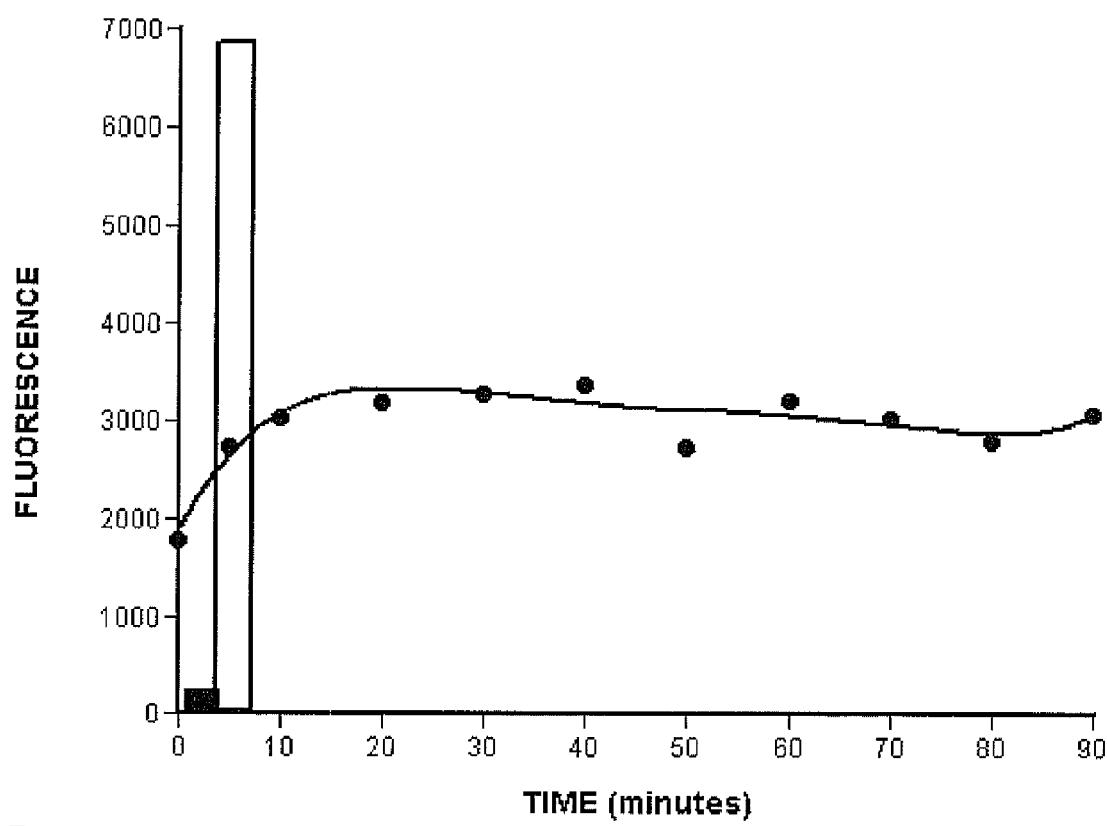


Figure 3

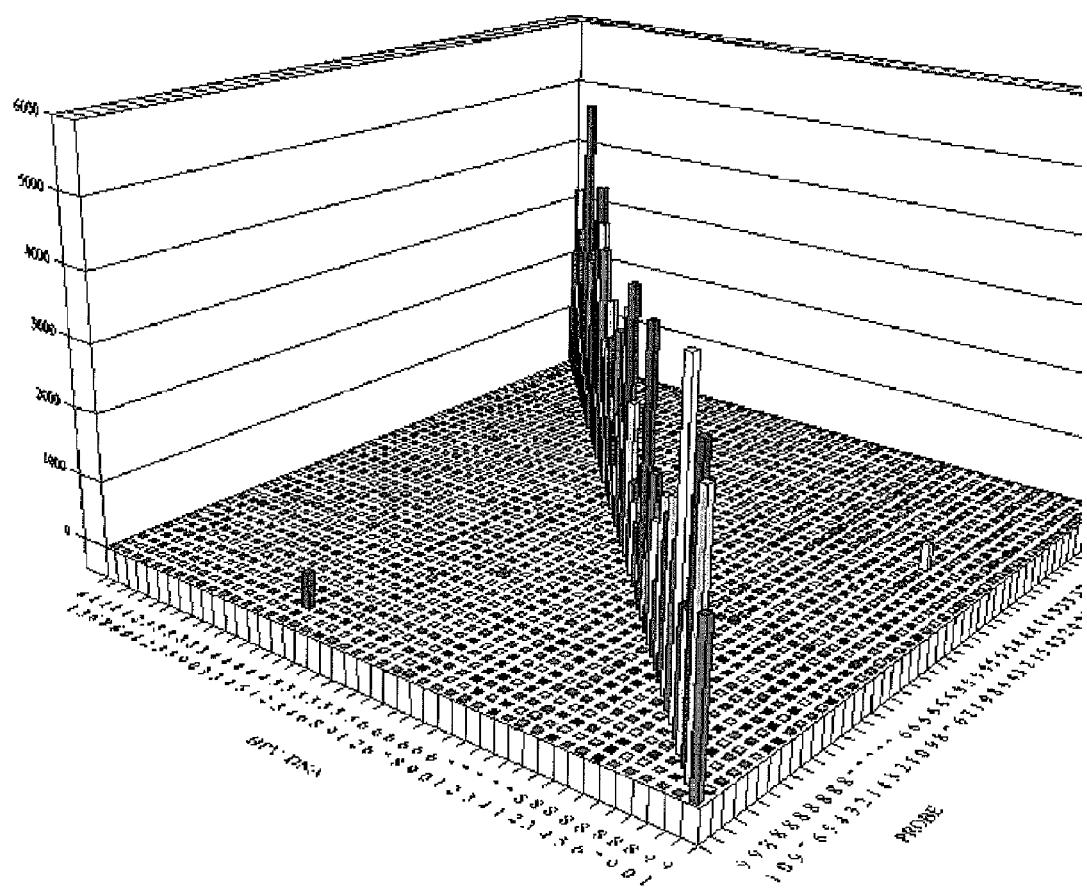


Figure 4

* * False negative or positive results

False Negatives

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**SET OF PROBES FOR THE DETECTION AND
TYPING OF 46 HUMAN PAPILLOMAVIRUS
MUCOSAL TYPES**

PRIOR APPLICATION INFORMATION

The instant application claims the benefit of U.S. Provisional Patent Application 61/296,245 filed Jan. 19, 2010

FIELD OF THE INVENTION

The present invention relates to reagents and methods for genotyping human papillomaviruses (HPV). In particular, the present invention relates to testing clinical samples for the type of HPV infection using a multiplex assay based on PCR amplification and detection using microspheres.

BACKGROUND OF THE INVENTION

Human papillomaviruses cause ubiquitous infectious of the keratinised epithelia of the skin and of the mucosae. About 120 HPV types have been characterized so far, which differ in prevalence, epidemiology and clinical manifestations (de Villiers et al., 2004). In particular, mucosal types infect the keratinised epithelia of the genital, anal and oro-pharyngeal mucosae (Muñoz et al., 2003; Muñoz and Bosch, 1997; Van Ranst et al., 1992; Chan et al., 1995, D'Souza et al., 2007, Bosh et al., 2008). Mucosal HPVs are most commonly transmitted by sexual contact, and infect sexually active people with a very high prevalence. It is estimated that the lifetime incidence of HPV infection in women is 80% (Bekkers et al., 2004), and the overall prevalence of active infection worldwide varies from 1.4% to 25% (Clifford et al., 2005).

Although the vast majority of infections are benign and self-limiting, a subset of "high risk" HPV types have the potential to cause persistent infection that may progress to malignant transformation and invasive cancer (Muñoz et al., 2003). Cervical cancer is the most common HPV-associated malignancy and it is now clear that HPV is a necessary cause of virtually all cervical cancers (Bosch and Muñoz, 2002; zur Hausen, 2002, Bosch et al., 2002; Muñoz et al., 2003; Walboomers et al., 1999, Smith et al., 2007). HPV associated malignancies are also found in the anal canal (Melbye and Sprogel, 1991; Palefsky et al., 1991), vulva (Buscema et al., 1988), the penis (Gregoire et al., 1995; Iwasawa et al., 1993), oro-pharyngeal mucosae and other head and neck tissues (D'Souza, et al., 2007; Mork et al., 2001; Gillison et al., 2000; Syrjanen, 2005).

Since HPV infection is necessary for the development of virtually all cervical cancers, detection of high risk HPV types is being considered as a screening method for cervical cancer, alongside, or even in substitution of, traditional cytological screening using the Papanicolaou methods (pap test), with the promise of improving the sensitivity and cost effectiveness of cervical cancer screening programs (Cuzick et al., 2008; Cuzick et al., 2003; Ronco et al., 2006; Schiffman et al., 2005; Kim et al., 2005; Davies et al., 2006; Mayrand et al., 2006; Cuzick et al., 2006).

Two type-specific HPV vaccines (Gardasil, from Merck-Frosst for types 16, 18, 6 and 11; Cervarix from Glaxo-Smith-Kline for types 16 and 18) have recently been developed and clinical trials have shown that they are extremely effective in preventing both persistent infection with HPV and the dysplastic changes in the cervical epithelium that lead to malignant transformation (Koutsy et al., 2002; Villa et al., 2005; Harper et al., 2004; Harper et al., 2006). However, since vaccines are type-specific it is important to know the distri-

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bution of the various HPV types in a population, as well as to have a surveillance system in place to monitor vaccine efficacy and unexpected shifts in the frequency of HPV types not covered by the vaccines.

It is therefore expected that the routine use of type-specific tests for HPV will become more widespread, outside their current use in epidemiological studies for research purposes.

Currently, typing of HPV requires amplification by various PCR methods, followed by detection of specific sequences using either direct sequencing of the PCR products, RFLP methods (many methods have been described in the literature, for example Lungo et al., 1992; Menzo et al., 2008, Nobre et al., 2008; Santiago et al., 2006), Southern blot or dot blot using specific probes (for example Gregoire et al., 1989; Josefsson et al., 1999), reverse line hybridization (Gravitt et al., 1998; Kleter et al., 1999; van der Brule et al., 2002; Melchers et al., 1999), DNA microarray methods (Min et al., 2006; Albrecht et al., 2006; Choi et al., 2003; Huang et al., 2004; Hwang et al., 2003; Oh et al., 2004; Nuovo et al., 2008), and others (for example Nishiwaki et al., 2008; Dell'atti, 2007; Gao et al., 2003; Gharizadeh et al., 2007; Han et al., 2006; Lee et al., 2005; Liu et al., 2003; Zhang et al., 2003). In particular, reverse line blot methods have been validated and have been used extensively for epidemiological studies. Two leading commercial genotyping methods, InnoLiPA (van Hamont, 2006) and Roche linear array (Coutlee et al., 2006), are based on the reverse hybridization technology. The Roche Linear Array genotyping kit as been approved by FDA and it is the leading commercial genotyping method.

However, these methods are not suitable for high throughput testing and they rely on a subjective visual assessment of band intensity for determining the results.

The xMAP technology developed by Luminex (Austin, Tex., USA) is based on microspheres that can be produced in 100 different "colours" depending on the ratio of two spectrally distinct fluorophores coupled to the microspheres. The different colours can be recognized by flow cytometers and the different type of microspheres can be enumerated and analyzed for the presence of specific bound ligands. This technology has been the basis for a variety of multiplex assays for serology, genotyping and other analytical applications. A description of the Luminex technology and a list of publications can be found at the Luminex web site.

Each type of microsphere can be coupled with a specific ligand, e.g. with DNA probes specific for each type of HPV in this work, and mixed together to form a multiplex assay. The PCR products derived from HPV samples are labelled with biotin and mixed with the beads carrying the probes, so that HPV DNA will hybridize with the cognate probe. The flow cytometer will then sort the different "coloured" microspheres and determine which type carries the fluorescence due to the HPV DNA. The computer software driving the flow cytometer will indicate which beads are fluorescent, thereby identifying the HPV type(s) present in the sample. The advantages of this method is the low cost per assay, the possibility of automation for a high throughput assay, and the flexibility derived from the possibility of adding or removing types of microspheres depending on the need of the assay or on the discovery of new types. Several microsphere-based multiplex assay for HPV genotyping have been published. The method by Wallace et al. (2005) is a multiplex microsphere assay with probes for 45 mucosal HPV. However, formal validation was performed for only a few types and only 20 types were detected from clinical samples, without independent validation of the genotyping result. The method published by Oh et al. (2007) detects 15 HPV types and it has been validated against a 132 clinical samples. A 56 sample comparison with

a DNA microarray genotyping method is also shown. The method, by Schmitt et al. (2006), has been carefully validated with HPV plasmids and clinical samples and covers the 22 most common mucosal HPV types. The method by Jiang et al. (2006) describes specific probes for 26 HPV mucosal types. Validation was performed with synthetic oligonucleotides complementary to the probes and with a limited number of clinical samples. A commercial method developed by Qiagen (Hilden, Germany) is able to type 18 HPV high-risk using a proprietary set of primers, followed by detection using a Luminex system. At least one study comparing this Luminex Qiagen test to a reverse line blot hybridization has been published (Seme et al., 2009).

Herein, we report the design of novel HPV type-specific probes and the development of a microsphere multiplex assay that can detect 46 different mucosal types in a single reaction. In addition the unique probe set, compared to the previous method we introduce 2 innovations: i) the use of longer probes (30 mers) to provide for a greater specificity for variants and closely related types; ii) the production of single stranded DNA products by selective digestion of the PCR products with exonuclease, which produces a greater signal to noise ratio, making a washing step unnecessary.

SUMMARY OF THE INVENTION

We have described a set of 46 DNA probes and a PCR amplification method for the detection of 46 mucosal HPV types using the Luminex xMAP technology. This technology uses a mixture of sortable microsphere coupled to the specific HPV probes, so that all the 46 types can be detected simultaneously in one reaction tube.

Our data shows that all the probes are sensitive and specific for the detection of the 46 HPV types, without cross-hybridization. This conclusion is supported by the use of reference DNA from the 46 types and an extensive validation using direct sequencing as a gold standard for the identification of the HPV types.

Amplified DNAs from at least 32 HPV types can be detected simultaneously and precisely by this Luminex method.

Comparison with a leading commercial HPV typing method, the Roche Linear Array, confirms that the NML Luminex method is suitable for the identification of HPV types in clinical samples containing 3 or less HPV types. However, the PCR amplification method is less efficient in amplifying DNA from samples with multiple infections containing 4 or more HPV types. This is a problem caused by the PCR amplification method and not by the set of probes or the Luminex detection system. The less efficient amplification in multiple infections is a significant problems for HPV types 52, 53, 61, 73 84 and 89 but not for the major oncogenic HPV types, which are most important in epidemiology and clinical practice.

When samples with 4 or more HPV types are excluded, detection by NML Luminex and Roche Linear array are equivalent. Therefore, use of the NML Luminex method on populations with high frequency of multiple infections (such as HIV patients, men who have sex with men or sex workers) will lead to an underestimation of the prevalence with certain types. On the contrary, use of the NML Luminex method on a general population of women, where the prevalence of infections with 4 or more types is negligible, will produce accurate prevalence results for most types.

The NML Luminex HPV genotyping method has the advantage of detecting almost all genital HPV types and of being very sensitive thanks to the nested PCR method. The

Luminex xMAP technology allows for a very quick, hands-off reading of the samples and an objective computational interpretation of the results. Because our method has no washing steps or visual reading steps, it is easily amenable to automation.

According to a first aspect of the invention, there is provided a method of detecting and typing a human papilloma virus (HPV) type infection in a sample comprising:

- a) providing a sample suspected of comprising at least one HPV type;
- b) adding to the sample primers suitable for amplifying the L1 region of HPV;
- c) incubating the sample under conditions suitable for DNA amplification;
- d) adding at least one probe having a nucleotide sequence as set forth in any one of SEQ ID NOs: 1-46, said probe binding to only one HPV type under hybridization conditions, each said at least one probe further comprising a unique tag;
- e) incubating said probe and said sample under conditions suitable for hybridization; and
- f) detecting hybridization of at least one said tagged probe.

According to a second aspect of the invention, there is provided a set of probes for detection and typing human papilloma virus (HPV) types, each said probe of said set hybridizing to only one HPV type under hybridizing conditions, each said probe of said set consisting of a unique tag and a nucleotide sequence as set forth in one of SEQ ID NOs: 1-46.

According to a third aspect of the invention, there is provided a set of probes for detection and typing human papilloma virus (HPV) types, each said probe of said set hybridizing to only one HPV type under hybridizing conditions, each said probe of said set consisting of a unique tag and a nucleotide sequence as set forth in one of SEQ ID NOs: 1, 2, 4 or 5.

According to a fifth aspect of the invention, there is provided a set of probes for detection and typing human papilloma virus (HPV) types, each said probe of said set hybridizing to only one HPV type under hybridizing conditions, each said probe of said set consisting of a unique tag and a nucleotide sequence as set forth in one of SEQ ID NOs: 4, 5 and 17.

According to a sixth aspect of the invention, there is provided a set of probes for detection and typing human papilloma virus (HPV) types, each said probe of said set hybridizing to only one HPV type under hybridizing conditions, each said probe of said set consisting of a unique tag and a nucleotide sequence as set forth in one of SEQ ID NOs: 4, 5, 8, 10, 11, 12, 17, 18, 19, 22, 23, 24, 27 and 29.

According to a seventh aspect of the invention, there is provided a set of probes for detection and typing human papilloma virus (HPV) types, each said probe of said set hybridizing to only one HPV type under hybridizing conditions, each said probe of said set consisting of a unique tag and a nucleotide sequence as set forth in one of SEQ ID NOs: 6, 4, 5, 7, 8, 10, 11, 12, 17, 18, 19, 20, 22, 23, 24, 27, 28, 29, 30, 31, 34, 37, 40 and 46.

According to an eighth aspect of the invention, there is provided a set of probes for detection and typing human papilloma virus (HPV) types, each said probe of said set hybridizing to only one HPV type under hybridizing conditions, each said probe of said set consisting of a unique tag and a nucleotide sequence as set forth in one of SEQ ID NOs: 1, 2, 9, 13, 14, 15, 16, 21, 25, 26, 27, 28, 29, 30, 31, 33, 35, 36, 38, 39, 41, 42, 43, 44 and 45.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1—Preliminary hybridization tests using 20 mer probes for HPV 6 and HPV 16—A mixture of two types of

microspheres coupled with 20 mer probes for HPV 6 and 16 (as described in Table 1) were hybridized with the indicated volume of PCR reaction (panel A and B), with a 20 mer oligonucleotide exactly complementary to the HPV 16 probe (panel C), or with an unrelated PCR product (β -globin DNA).

FIG. 2—Position of the probes for the 46 HPV types considered in this submission. The alignment of the L1 region comprised between the GP5+/GP6+ primers is shown. The positions of the primers are indicated by the boxes while the probe sequences are in bold and underlined. Shown are probes for HPV 6 (SEQ ID NO: 231), HPV 11 (SEQ ID NO: 232); HPV 13 (SEQ ID NO: 233), HPV 16 (SEQ ID NO: 234), HPV 18 (SEQ ID NO: 235), HPV 26 (SEQ ID NO: 236), HPV 30 (SEQ ID NO: 237), HPV 31 (SEQ ID NO: 238), HPV 32 (SEQ ID NO: 239), HPV 33 (SEQ ID NO: 240), HPV 35 (SEQ ID NO: 241), HPV 39 (SEQ ID NO: 242), HPV 40 (SEQ ID NO: 243), HPV 42 (SEQ ID NO: 244), HPV 43 (SEQ ID NO: 245), HPV 44 (SEQ ID NO: 246), HPV 45 (SEQ ID NO: 247), HPV 51 (SEQ ID NO: 248), HPV 52 (SEQ ID NO: 249), HPV 53 (SEQ ID NO: 250), HPV 54 (SEQ ID NO: 251), HPV 56 (SEQ ID NO: 252), HPV 58 (SEQ ID NO: 253), HPV 59 (SEQ ID NO: 254), HPV 61 (SEQ ID NO: 255), HPV 62 (SEQ ID NO: 256), HPV 66 (SEQ ID NO: 257), HPV 67 (SEQ ID NO: 258), HPV 68 (SEQ ID NO: 259), HPV 69 (SEQ ID NO: 260), HPV 70 (SEQ ID NO: 261), HPV 71 (SEQ ID NO: 262), HPV 72 (SEQ ID NO: 263), HPV 73 (SEQ ID NO: 264), HPV 74 (SEQ ID NO: 265), HPV 81 (SEQ ID NO: 266), HPV 82 (SEQ ID NO: 267), HPV 83 (SEQ ID NO: 268), HPV 84 (SEQ ID NO: 269), HPV 85 (SEQ ID NO: 270), HPV 86 (SEQ ID NO: 271), HPV 87 (SEQ ID NO: 272), HPV 89 (SEQ ID NO: 273), HPV 90 (SEQ ID NO: 274), HPV 91 (SEQ ID NO: 275) and HPV 97 (SEQ ID NO: 276).

FIG. 3—Effect of T7 exonuclease digestion of nested PCR products on hybridization to Luminex beads. HPV 16 DNA was amplified by MY09/My11 and GP5+/GP6+ nested PCR, as described above, and the products were digested with T7 exonuclease for the indicated times. After digestion, the PCR products were hybridized to Luminex beads carrying the HPV 16 probe and detected as described above. The GP6+ primer contained a 5' biotin moiety, for detection by the Luminex technology, and phosphorothioate bonds in the first 4 nucleotides on the 5', to protect this strand from the action of the T7 exonuclease. The black bar and the white bar represent the fluorescence signals of a negative sample and of a sample containing a biotinylated oligonucleotide complementary to the HPV 16 probe.

FIG. 4—Graphic representation of the data of Table 4—The probes are on the right axis and the HPV DNAs on the left axis. The vertical axis represents the fluorescence read for each microsphere carrying a specific HPV probe. The bars on the diagonal represent the hybridization of HPV DNA type with the intended cognate probe.

FIG. 5—Simultaneous detection of multiple HPV types—Samples containing DNA from increasing numbers of HPV types were prepared as described in the text and then detected by the NML Luminex method as described. The number of types in each sample is indicated in the leftmost column. The second column from the left indicates what additional HPV type was added to the mixture. A “+” sign indicates a positive result (over 50 FU). The asterisks indicate false positive or false negative results.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood

by one of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned hereunder are incorporated herein by reference.

DNA probes comprising SEQ ID NOs: 1 through 46 were generated according to the specific sequences of 46 types of genital HPV, namely type 6 (SEQ ID NO: 1), 11 (SEQ ID NO: 10 2), 13 (SEQ ID NO: 3), 16 (SEQ ID NO: 4), 18 (SEQ ID NO: 5), 26 (SEQ ID NO: 6), 30 (SEQ ID NO: 7), 31 (SEQ ID NO: 8), 32 (SEQ ID NO: 9), 33 (SEQ ID NO: 10), 35 (SEQ ID NO: 11), 39 (SEQ ID NO: 12), 40 (SEQ ID NO: 13), 42 (SEQ ID NO: 14), 43 (SEQ ID NO: 15), 44 (SEQ ID NO: 16), 45 (SEQ ID NO: 17), 51 (SEQ ID NO: 18), 52 (SEQ ID NO: 19), 53 (SEQ ID NO: 20), 54 (SEQ ID NO: 21), 56 (SEQ ID NO: 22), 58 (SEQ ID NO: 23), 59 (SEQ ID NO: 24), 61 (SEQ ID NO: 25), 62 (SEQ ID NO: 26), 66 (SEQ ID NO: 27), 67 (SEQ ID NO: 28), 68 (SEQ ID NO: 29), 69 (SEQ ID NO: 30), 70 (SEQ ID NO: 31), 71 (SEQ ID NO: 32), 72 (SEQ ID NO: 33), 73 (SEQ ID NO: 34), 74 (SEQ ID NO: 35), 81 (SEQ ID NO: 36), 82 (SEQ ID NO: 37), 83 (SEQ ID NO: 38), 84 (SEQ ID NO: 39), 85 (SEQ ID NO: 40), 86 (SEQ ID NO: 41), 87 (SEQ ID NO: 42), 89 (SEQ ID NO: 43), 90 (SEQ ID NO: 44), 91 (SEQ ID NO: 45) and 97 (SEQ ID NO: 46). In order to make each probe sensitive and specific, the probes were tested in a multiplex assay as described below. Probes that in these tests did not hybridize to the intended HPV type or that cross-hybridized to other types were re-designed, sometimes repeatedly, until all probes hybridized to unique HPV type DNA. Accordingly, each respective probe binds specifically to only one specific HPV genome or HPV type. The history of the probe design is shown in Table 2.

For the multiplex assay, each probe was conjugated to one 35 of 46 types of fluorescent microspheres, each with different ratios of red and infrared fluorophores, according to the manufacturers instructions. The micropsheres produced by 40 Luminex Corp. are colour coded with a combination of two fluorescence dyes into 100 different sets that can be recognized and counted by a flow cytometer using a red laser. The 45 flow cytometer can also detect a reporter dye bound to any set of beads using a separate green laser. For this embodiment, 46 sets of beads were selected and each set was coupled to a unique 30mer oligonucleotide probe designed to hybridize sensitively and specifically to one of 46 types of genital HPV DNA, amplified as described below. The 46 sets of beads were mixed to constitute a multiplex reaction that could detect any combination of the 46 types of HPV DNA present in clinical specimens.

The probes were designed to amplify the region comprised 50 between the PCR amplification primers GP5+/GP6+. This region is 141 bp long for HPV 16 (nucleotides 6624 to 6764, GenBank accession no. AF125673), but varies in length slightly depending on the HPV type. GP5+/GP6+ are general 55 primers that amplify the DNA from most HPV types. Published primers sets MY09/My11 and primer set PGMY are also general primers which amplify most genital HPV types. They are situated outside the GP+/GP6+ region and therefore they can be used for a nested PCR reaction with the GP5+/GP6+ primers, in order to improve the sensitivity and the spectrum of HPV types that can be amplified, especially when multiple types are present in the same sample.

Using these primers, HPV DNA from clinical samples was 60 amplified and then treated with T7 exonuclease to produce a single stranded, biotin labelled DNA complementary to the 65 probes coupled to the microspheres. The single stranded HPV DNA and the tagged microspheres were then co-incubated, so

that the HPV DNA could bind to its cognate probe on the microspheres. Streptavidin conjugated to the fluorophore phycoerythrine was then added. Streptavidin binds tightly to biotin conferring phycoerythrine fluorescence to those microsphere that are bound to HPV DNA. The samples were then analyzed by flow cytometry which provided an analysis of the numbers of each type of bound microspheres and their level of phycoerythrine fluorescence. High phycoerythrine fluoresce on specific beads indicates the presence of HPV DNA of specific types.

In addition to the multiplex assay for 46 HPV types, the microspheres can be mixed in different combinations to test separately only for HPV types contained in vaccines (HPV type 6 (SEQ ID NO: 1), 11 (SEQ ID NO: 2), 16 (SEQ ID NO: 4) and 18 (SEQ ID NO: 5)), or to test for the most malignant HPV types (HPV types 16 (SEQ ID NO: 4), 18 (SEQ ID NO: 5) and 45 (SEQ ID NO: 17)), or for the most common HPV types (HPV type 16 (SEQ ID NO: 4), 18 (SEQ ID NO: 5), 31 (SEQ ID NO: 8), 33 (SEQ ID NO: 10), 35 (SEQ ID NO: 11), 39 (SEQ ID NO: 12), 45 (SEQ ID NO: 17), 51 (SEQ ID NO: 18), 52 (SEQ ID NO: 19), 56 (SEQ ID NO: 22), 58 (SEQ ID NO: 23), 59 (SEQ ID NO: 24), 66 (SEQ ID NO: 27) and 68 (SEQ ID NO: 29)), or to test for all oncogenic HPV types (HPV type 26 (SEQ ID NO: 6), 16 (SEQ ID NO: 4), 18 (SEQ ID NO: 5), 30 (SEQ ID NO: 7), 31 (SEQ ID NO: 8), 33 (SEQ ID NO: 10), 0.35 (SEQ ID NO: 11), 39 (SEQ ID NO: 12), 45 (SEQ ID NO: 17), 51 (SEQ ID NO: 18), 52 (SEQ ID NO: 19), 53 (SEQ ID NO: 20), 56 (SEQ ID NO: 22), 58 (SEQ ID NO: 23), 59 (SEQ ID NO: 24), 66 (SEQ ID NO: 27), 67 (SEQ ID NO: 28), 68 (SEQ ID NO: 29), 69 (SEQ ID NO: 30), 70 (SEQ ID NO: 31), 73 (SEQ ID NO: 34), 82 (SEQ ID NO: 37), 85 (SEQ ID NO: 40), and 97 (SEQ ID NO: 46)), or to test only for non-oncogenic (low risk) types (6 (SEQ ID NO: 1), 11 (SEQ ID NO: 2), 32 (SEQ ID NO: 9), 40 (SEQ ID NO: 13), 42 (SEQ ID NO: 14), 43 (SEQ ID NO: 15), 44 (SEQ ID NO: 16), 54 (SEQ ID NO: 21), 61 (SEQ ID NO: 25), 62 (SEQ ID NO: 26), 66 (SEQ ID NO: 27), 67 (SEQ ID NO: 28), 68 (SEQ ID NO: 29), 69 (SEQ ID NO: 30), 70 (SEQ ID NO: 31), 72 (SEQ ID NO: 33), 74 (SEQ ID NO: 35), 81 (SEQ ID NO: 36), 83 (SEQ ID NO: 38), 84 (SEQ ID NO: 39), 86 (SEQ ID NO: 41), 87 (SEQ ID NO: 42), 89 (SEQ ID NO: 43), 90 (SEQ ID NO: 44) and 91 (SEQ ID NO: 45)).

In accordance with a first embodiment of the present invention, there is provided a series of DNA probes that can be used in conjunction with DNA amplification techniques to genotype various strains of HPV.

In a second embodiment of the invention, the series of DNA probes that can be used in a multiplexed format assay to simultaneously detect multiple strains of HPV.

In a third embodiment of the invention, the DNA probes can be used with other detection systems including Southern or Northern blots, reverse line blot hybridization, DNA microarray or ELISA, or other such systems as will be obvious to those skilled in the art.

According to an aspect of the invention, there is provided a method of detecting and typing a human papillomavirus (HPV) type infection in a sample comprising:

- a) providing a sample suspected of comprising at least one HPV type;
- b) adding to the sample primers suitable for amplifying the L1 region of HPV;
- c) incubating the sample under conditions suitable for DNA amplification;
- d) adding at least one probe having a nucleotide sequence as set forth in any one of SEQ ID NOs: 1-46, said probe binding to only one

HPV type under hybridization conditions, each said at least one probe further comprising a unique tag;

e) incubating said probe and said sample under conditions suitable for hybridization; and

f) detecting hybridization of at least one said tagged probe.

As discussed herein, the sample may contain more than one HPV type and the 'at least one probe' may be a set of probes comprising or consisting of respective probes having nucleotide sequences as set forth in any one of SEQ ID NOs: 1-46 and a unique tag or identification tag which uniquely identifies the respective probe. For example, all probes having a nucleotide sequence as set forth according to SEQ ID NO: 1 will have the same tag as will all probes having a nucleotide sequence as set forth in SEQ ID NO: 2.

As discussed herein, the hybridization conditions are sufficiently stringent that the probe will bind only to the target DNA. For example, the hybridization conditions may be sufficiently stringent for hybridization of two strands to occur only if there is 15, 16, 17, 18, 19, 20 or more consecutive nucleotides having an exact match.

As will be appreciated by one of skill in the art, the probes consisting of nucleotide sequences as set forth in any one of SEQ ID NOs: 1-46 and a unique tag can be used together or in any sub-combination thereof in a multiplex assay to specifically type HPV types in a given sample. Specifically, because each probe has a unique tag associated therewith, hybridization of a respective probe to a DNA molecule within the sample indicates the presence of the corresponding HPV type in that sample. The probe set is unique in that the probes do not cross-hybridize, as discussed below.

In some embodiments, at least one probe may refer to a mixture of probes, each representative probe of said mixture having a nucleotide sequence as set forth in SEQ ID NOs: 1, 2, 4 or 5 or as set forth in SEQ ID NOs: 4, 5 or 17 or as set forth in SEQ ID NOs: 4, 5, 8, 10, 11, 12, 17, 18, 19, 22, 23, 24, 27 or 29 or as set forth in SEQ ID NOs: 6, 4, 5, 7, 8, 10, 11, 12, 17, 18, 19, 20, 22, 23, 24, 27, 28, 29, 30, 31, 34, 37, 40 or 46 or as set forth in SEQ ID NOs: 1, 2, 9, 13, 14, 15, 16, 21, 25, 26, 27, 28, 29, 30, 31, 33, 35, 36, 38, 39, 41, 42, 43, 44 or 45.

The unique tag is a combination of two fluorescent dyes.

The unique tag is a combination of different ratios of red and infra-red fluorophores, as discussed herein.

According to another aspect of the invention, there is provided a set of probes for detection and typing human papilloma virus (HPV) types, each said probe of said set hybridizing to only one HPV type under hybridizing conditions, each said probe of said set consisting of a unique tag and a nucleotide sequence as set forth in one of SEQ ID NOs: 1-46.

According to another aspect of the invention, there is provided a set of probes for detection and typing human papilloma virus (HPV) types, each said probe of said set hybridizing to only one HPV type under hybridizing conditions, each said probe of said set consisting of a unique tag and a nucleotide sequence as set forth in one of SEQ ID NOs: 1, 2, 4 or 5. As will be appreciated by one of skill in the art, additional probes having sequences as set forth in any one of SEQ ID NOs: 3 and 6-46 and any combination thereof may be added to the probe set.

According to another aspect of the invention, there is provided a set of probes for detection and typing human papilloma virus (HPV) types, each said probe of said set hybridizing to only one HPV type under hybridizing conditions, each said probe of said set consisting of a unique tag and a nucleotide sequence as set forth in one of SEQ ID NOs: 4, 5 and 17.

According to another aspect of the invention, there is provided a set of probes for detection and typing human papil-

loma virus (HPV) types, each said probe of said set hybridizing to only one HPV type under hybridizing conditions, each said probe of said set consisting of a unique tag and a nucleotide sequence as set forth in one of SEQ ID NOs: 4, 5, 8, 10, 11, 12, 17, 18, 19, 22, 23, 24, 27 and 29.

According to another aspect of the invention, there is provided a set of probes for detection and typing human papilloma virus (HPV) types, each said probe of said set hybridizing to only one HPV type under hybridizing conditions, each said probe of said set consisting of a unique tag and a nucleotide sequence as set forth in one of SEQ ID NOs: 6, 4, 5, 7, 8, 10, 11, 12, 17, 18, 19, 20, 22, 23, 24, 27, 28, 29, 30, 31, 34, 37, 40 and 46.

According to another aspect of the invention, there is provided a set of probes for detection and typing human papilloma virus (HPV) types, each said probe of said set hybridizing to only one HPV type under hybridizing conditions, each said probe of said set consisting of a unique tag and a nucleotide sequence as set forth in one of SEQ ID NOs: 1, 2, 9, 13, 14, 15, 16, 21, 25, 26, 27, 28, 29, 30, 31, 33, 35, 36, 38, 39, 41, 42, 43, 44 and 45.

EXAMPLES

Oligonucleotides

Oligonucleotides were synthesized at the DNA Core Section of the National Microbiology Laboratory. The probes carried a 5' C₁₂ amino linker modification for coupling to the carboxyl group of the Luminex microspheres. The MY09, MY11, GP5+ and the modified GP6+ primer for the PCR amplification of HPV DNA, were purchased from Invitrogen (Burlington ON, Canada).

PCR amplification

HPV DNA from plasmid or clinical specimens was amplified by a nested PCR method using the MY09/MY11 primers for the first step (Manos et al., 1989) and GP5+/GP6+ primers for the second step (Roda Husman et al., 1995). For optimal amplification of clinical samples with multiple HPV types, PGMY primers were used for the first step (Gravitt et al., 2000). The GP6+ primer carried the following modification: i) a 5' biotin label to be used as a ligand for the streptavidin-PE for detection of PCR products (See below); ii) the first 4 nucleotides on the 5' end were linked by phosphorothioate bonds to confer resistance to the action of the bacteriophage T7 gene 6 exonuclease (See below and in the result section). PCR amplification was performed in 1xPCR Buffer (Invitrogen, Cat #10342-020) in the presence of 4 mM MgCl₂, 200 μM of dNTP (Invitrogen, Cat#10297-018), 0.2 mM of each primer and 1.25 U of AmpliTaq Gold polymerase (Applied Biosystem, Cat #4311816). The first round of nested PCR amplification with the MY09/MY11 primers started with a 5 min initial denaturation step at 94° C., followed by 30 cycles of denaturation at 94° C. for 30 seconds, annealing at 55° C. for 30 seconds and elongation at 72° C. for 60 seconds, followed by a 7 min final extension at 72° C. Amplification with PGMY primers was carried out for 40 cycles (denaturation at 94° C. for 30 sec, annealing 55° C. for 30 sec, elongation 72° C. for 30 sec) in the presence of 6 mM MgCl₂, 200 μM dNTPs and 0.6 μM each of the 18 primers comprising the PGMY mixture (Gravitt et al., 2000). One to 5 (typically 2) μL of this reaction were used for the second round of amplification with GP5+/GP6+ primers under the following conditions: 5 min initial denaturation at 94° C., followed by 30 cycles of 94° C. for 30 seconds, 40° C. for 20 seconds and 72° C. for 30 seconds, followed by a 7 min final extension at 72° C. One-step PCR with GP5+/GP6+ primers was con-

ducted under the following conditions: a 5 min initial denaturation at 94° C. followed by 30 cycles of 94° C. for 30 seconds, 40° C. for 20 seconds and 72° C. for 30 seconds followed by a 7 min final extension at 72° C.

5 Digestion of PCR Products with T7 Exonuclease

After PCR, the GP5+ strand complementary to the biotinylated strand, was removed by digestion with T7 genes exonuclease, a 5'-3' processive exonuclease. The other strand was protected from the action of T7 exonuclease by the 4 phosphorothioate bonds on the 5' (Nikiforov et al, 1994). This digestion produced a single stranded, biotin labelled DNA complementary to the probes coupled to the Luminex beads and it was performed by adding T7 exonuclease (New New England Biolabs, Cat# M0263L) to PCR products at a final concentration of 0.4 U/μL. The reaction was stopped by adding 0.5M EDTA at a final concentration of 12.5 μL of 0.5M EDTA.

Preparation of Microspheres

Microspheres labelled with different ratios of red and infrared fluorophores were purchased from Luminex (Austin, Tex., USA, Cat #L100-CXXX-01) and coupled to HPV type-specific probes carrying a 5' amino modification that reacts with the carboxyl groups on the microspheres following the instruction of the manufacturer with minor modifications.

20 Briefly, the microsphere stock (Luminex) was vortexed vigorously then an aliquot containing 5.0×10⁶ microspheres from each set was placed in a separate 1.5 ml microfuge tube, resuspended in a in a sonicating water bath (Branson) and centrifuged at 14000×g for 2 min. The supernatant was 25 removed and the microshperes were resuspended in 50 μL of 0.1 M 2-N-morpholinoethansulfonic acid (MES) (Sigma Cat #M-2933) at a pH of 4.5. Then 1 μL of a 1 mM solution of the appropriate type of amino substituted oligonucleotide was added to a different set of microspheres and 2.54 of a 10 mg/mL solution of 1-Ethyl-3-3-dimethylaminopropyl carbodiimide HCl (EDC) (Fisher Cat #22980) were added to each 30 tube. The tubes were vortexed and, after an incubation of 30 min at RT in the dark, 2.5 μL of 10 mg/ml EDC were added to 35 each tube and incubated in the dark for 30 minutes. After the 40 second incubation period 1 ml of 0.02% Tween 20 (Sigma Cat # P-9416) was added and the tubes were centrifuged for 2 minutes at 14,000×g. The supernatant was removed and 1 ml of 0.1% SDS (sodium dodecyl sulfate) was added to the 45 microsphere pellet, the tubes were vortexed and then microcentrifuged for 2 minute at 14,000×g. The supernatant was removed and the pellet was resuspended in 100 μL of TE. The microspheres coupled to the probes were stored in the dark at 4° C. for a maximum of 6 months.

Luminex Assay

50 For the Luminex assay typically 15 microspheres/μL of each set were mixed in a reaction mixture. Exonuclease-digested PCR products were placed in a 96 well PCR microplate (Fisher, Cat # CS006509) in a total volume of 17 μL and sealed with a 96 well sealing cover (Fisher, Cat # CS006555).

55 The microplate was incubated at 95° C. for 10 minutes to denature the DNA and 33 μL of the microsphere mix was added. The samples were incubated at the hybridization temperature of for 10 min and, after addition of 25 μL of a 0.04 mg/μL solution of streptavidine-phycoerythrin (Invitrogen Cat # S-866) in 1x tetramethyl ammonium chloride (TMAC) (Sigma, Cat # T-3411) was added to the samples and incubated for 5 more minutes at 60° C. Samples were analyzed on a Luminex Liquid Chip 200 flow cytometer using the Luminex IS software. The analysis was carried out at 60° C. 60 with a maximum volume of 50 μL of sample and a minimum count of 100 microspheres per type, with a setting of 8,300 and 16,500 for the lower and upper gate, respectively.

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Example 1

Design and Selection of Probes

The probes were targeted at the region of the L1 gene comprised between the GP5+/GP6+ primers (Roda Husman et al., 1995). This is a relatively poorly conserved region bracketed by two conserved regions where the GP5+/GP6+ primers bind. The length of this segment varies slightly among different types and, for example, it is 141 bp long in HPV16, corresponding to nt 6624 to 6764 of the sequence published by Flores et al., 1999 (GenBank accession no. AF125673).

Previous literature on the use of Luminex Xmap technology for detecting DNA sequence typically reported the use of 20 nt long probes. We therefore designed first 20 nt long probes, using the ArrayDesigner computer software (Premier BioSoft International) (Table 1), but preliminary experiments with probes and DNA from HPV type 6 and 16 showed that these probes were not sensitive for the detection of HPV DNA under our conditions. As shown in FIG. 1, DNA amplified from HPV 6 and HPV 16 clones failed to hybridize to the microsphere carrying the cognate 20 mer probe (Panel A and B). A biotinylated oligonucleotide exactly complementary to the HPV 16 probe did produce a considerable fluorescence of the HPV 16 microsphere but it also non-specifically increased the fluorescence of the HPV 6 microsphere (panel C).

Therefore, the probes were then re-designed as 30mers by adding 10 nt to the left or the right of the original probe. Longer probes also provide greater specificity and a better chance of discriminating among closely related HPV types or variants, for example HPV16 and HPV 31. This initial set of 30mers contained numerous unsuitable probes, either because they were cross-reactive (poor specificity) or because they were not binding efficiently to the intended target (poor sensitivity), or both. Unsuitable probes were redesigned typically by shifting their position 10 nucleotides to the right or to the left along the variable region of the GP5+/GP6+ fragment. This process was repeated until all probes were both specific and sensitive for the intended target. Attempts to predict the efficiency and specificity of the probes or to weed out probes with hairpins or other cross-reactive sequences proved ineffectual, because often probes behaved in an unexpected manner.

The history of the development of the probes is shown of Table 2, while the final sets of probes used for this method is shown on Table 3. FIG. 2 shows the location of the probes on the aligned sequences of the 46 HPV types covered by this method.

Example 2

Effect of Exonuclease

Simple denaturation of the double-stranded PCR products followed by hybridization to the probes on the microspheres produced a fluorescence signal that was much lower compared to the signal produced by hybridizing the microspheres to biotin-labelled single-stranded oligonucleotides (FIG. 3). We suspected that rehybridization of the long strands of the PCR products might have been thermodynamically more favourable than the hybridization of the GP6+ strand to the short (30 nt) probe physically constrained on the microsphere. We therefore decided to remove the non-labelled strand of the PCR product using bacteriophage T7 gene 6 exonuclease, according to the method described earlier (Nikiforov et al., 1994). T7 exonuclease is a 5'→3' processive

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enzyme that rapidly degrades one of the strand on a duplex DNA molecule (Kerr and Sadowski, 1972). In order to protect the GP6+ strand, carrying the biotin label, and selectively digest only GP5+ strand, the first 4 nucleotides at the 5' end of the molecule were modified to include phosphorothioate bonds between the deoxyribose moieties, instead of the usual phosphodiester bonds. This chemical modification is known to inhibit the action of T7 exonuclease, that can no longer digest the DNA molecule starting from such modified end (Nikiforov et al., 1994).

Optimal digestion conditions were determined by incubating 40 units of T7 exonuclease with 100 ul of PCR product for various times, and then measuring the fluorescence on the Luminex system. These experiments, like the one showed in FIG. 3 determined that an incubation of 40 minutes is optimum for the sensitivity of the test and increased the fluorescence signal by about 2 fold.

Example 3

Typing of HPV

Specificity and sensitivity for each type was determined by adding PCR product from a known source of HPV, clones carrying the whole HPV genome, when available, or clones of the MY region of the genome amplified by PCR from clinical samples or synthesized using published genomic sequences (see Material and methods for a complete list). All clones were confirmed by direct sequencing and comparison with published HPV sequences.

Using the PCR amplification method, exonuclease digestion and microsphere hybridization described above, amplified HPV DNA from each type was hybridized to a mixture of the 46 types of microspheres carrying the 46 specific HPV probes. After hybridization, the microsphere mixture was analyzed by the Luminex LiquidChip 200 flow cytometer. Four negative controls, containing only host cell DNA, were run alongside the samples. The average background fluorescence of each bead in the controls was subtracted from the fluorescence of each bead of the samples. This type of background correction is necessary because different bead types may have different background fluorescence. This correction avoids the need for a bead washing step, used in other Luminex procedures (Wallace et al., 2005; Oh et al., 2007; Schmitt et al., 2006; Jiang et al., 2006; Seme et al., 2009). A fluorescence signal greater than 100 FU after correction was chosen as threshold for positivity.

The complete results are shown in Table 4, where each column represents the fluorescence associated with the 50 microsphere carrying the probe for the indicated HPV type in the presence of the HPV DNA of the types indicated on the leftmost column. FIG. 4 shows the same results in graphic format. It can be seen that all the 46 probes strongly hybridize with the corresponding HPV DNA, but not with HPV DNA of different types. It should be noted that in the particular experiment shown in Table 4 and FIG. 4, the microsphere for HPV 89 also shows fluorescence above the 100 FU threshold level in the presence of HPV 44 DNA (513 FU), the microsphere for HPV 72 in the presence of HPV 81 DNA (118 FU) and the microsphere for HPV 44 in the presence of HPV 86 DNA (391 FU). This should be interpreted as random fluctuations, rather than systematic cross-reactivity, because the abnormal fluorescence reading was not reproducible in other experiments. This corresponds to a false positive rate of 3/1980 measurements or 0.15%. To avoid false positives, clinical samples are tested in duplicate and the measurement is repeated if the duplicates give discordant results.

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We then tested the ability of the Luminex method to detect infections with multiple types in the same sample, by amplifying DNAs from different HPV types separately and then mixing them together in a single Luminex detection reaction. The amount of DNA per type was kept constant, to simulate the situation of clinical samples, in which a mixture of different DNA is amplified to the maximum capacity of the PCR reaction, regardless of the number of types present. The results are presented in FIG. 5, that shows that at least 30 different types can be detected simultaneously with minimal cross hybridization. Some false negatives and false positives are however present. The false negatives are probably due to the fact that the fluorescence for each HPV type is low when many types are present and therefore some microsphere may fall under the 50 FU that was established as positivity threshold. False positive for HPV 72 are due to fluctuation in the background fluorescence of this microsphere.

Example 4

Validation Using Clinical Samples—Direct Sequencing

Validation against clinical samples was performed by comparing the results of the NML Luminex genotyping method with direct sequencing of the amplified products. Because direct sequencing identifies any HPV type without misclassification, this is a further test of the specificity of the probes of the NML Luminex assay.

Seven hundred seventy five samples were amplified by nested PCR as described above and the products were typed with the NML Luminex method. The same samples were amplified separately by nested PCR and run on an agarose gel to determine the presence of HPV DNA. Positive samples were sent for sequencing at the NML DNA Core facility, using GP5+ and GP6+ primers to sequence both strands of the amplified products. The assembled sequenced was compared against GenBank sequences using BLAST (Altschul et al., 1990). Type identification required a nucleotide identity greater than 90% on a fragment of at least 60 nucleotide in length.

The results presented in Table 5, show that the two methods were 97.7% concordant for the detection of HPV, regardless of type. The sensitivity and specificity of the NML Luminex method vs direct sequencing, taken as a gold standard, were 98.8% (97.1-99.6, 95% CI) and 96.4% (96.4-93.8, 95% CI), respectively.

When positive identification of HPV type is taken into consideration, the direct sequencing method could not determine the sequence of 34 positive samples, 32 of which were typed by the NML Luminex method. There was no agreement on the HPV type detected for 13 out of 429 samples positive with both methods (3.3%). The NML Luminex method detected a total of 793 HPV types, vs 577 for direct sequencing. This discrepancy is due to the fact that direct sequencing cannot detect multiple HPV types present in the same sample.

A breakdown of HPV types detected by the two methods is presented in Table 6.

From the validation against the direct sequencing method, it is impossible to establish if the extra types detected by the NML Luminex assay are due to better sensitivity for multiple infections or to poor specificity.

Example 5

Validation Using Clinical Samples—Comparison to Roche Linear Assay

Therefore we compared the performance of the NML Luminex assay using the Roche LinearArray HPV genotyp-

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ing method as the gold standard. The Linear Array kit can detect 37 different genotypes and its amplification system, based on the PGMY primers, is particularly efficient in amplifying multiple types. Linear Array is FDA approved and it is one of the standard methods used in the literature on HPV epidemiology.

For this comparison we used a set of 880 samples that were tested for HPV at the National Microbiology Laboratory in parallel by the Roche Linear Array kit, according to the instruction of the manufacturer, and by the NML Luminex genotyping method.

The Linear Array probe for HPV 52 cross-reacts with HPV type 33, 35 and 58. Therefore all Linear Array HPV 52 results were confirmed by real time PCR specific for HPV 52 as previously described (Coutlee et al., 2007). Linear Array contains a probe designated HPV 55, but according to the latest classification of HPVs (de Villier et al., 2004) the type 55 is considered a variant of HPV 44 and HPV 55 has been removed. Therefore in this work we used the HPV 44 designation. Roche Linear Array probes IS39 and CP6108 recognize types more recently designated as HPV 82 and HPV 89 respectively. The more recent designation was used in this work.

Table 7 shows a comparison of the NML Luminex with Roche linear array for the detection of positive samples for any HPV type. Discordant results are 7% overall and 5.2% of discordant samples tested positive with the NML Luminex but not with the linear array. This is due to the greater sensitivity of the nested PCR used for the NML Luminex method and to the detection of HPV types not present on the linear array set of probes.

Table 8 shows the comparison of the NML Luminex method with the Roche Linear array for the detection of all HPV types and multiple infections. The Roche Linear array detected considerably more types of HPV (1111 vs. 917), due to the better performance in samples with high numbers of multiple infections. This reduced performance for multiple infections is not due to the greater sensitivity of the nested PCR used for the NML Luminex method and to the detection of HPV types not present on the linear array set of probes.

Table 9 shows the comparison results for the individual types. Apart from the types not detected by the Roche Linear Array (HPV 13, 32,

74, 85, 86, 87, 90 and 91) the detection of HPV types 52, 53,

61, 73 84 and 89 was statistically significantly more sensitive (χ^2 test) in the Roche linear Array, while the detection of

HPV type 67 was more sensitive in the NML Luminex.

Table 10 shows the results after exclusion of samples with multiple infections with 4 or more types, as determined by the Roche linear array. This Table shows a much better concordance between NML Luminex and Roche Linear Array with respect of total number of types detected (535 vs 534, respectively) and type breakdown. In addition to the types not detected by the Roche Linear Array, only type 52 (better detection for Linear Array) and type 67 (better detection for NML Luminex) are now significantly different.

While the preferred embodiments of the invention have been described above, it will be recognized and understood that various modifications may be made therein, and the appended claims are intended to cover all such modifications which may fall within the spirit and scope of the invention.

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TABLE 1

	Sequences		
	Sequence Definition	Probe Sequence	SEQ ID NO:
5	HPV 6	ACCACACGCAGTACCAAACAT	47
10	HPV 6	CATGCGTCATGTGGAAGAGT	48
	HPV11	ATGCGCCATGTGGAGGAGTT	49
	HPV11	TGGTAGATAACCACACGCAGT	50
	HPV13	TGACTGTGTGAGGCCACT	51
	HPV13	GTTGATACTACACGCAGTAC	52
	HPV16	ACCTACGACATGGGGAGGAA	53
15	HPV16	ATGTCATTATGTGCTGCCAT	54
20	HPV18	CAGTCTCCTGTACCTGGCA	55
	HPV18	AGATACCACTCCAGTACCA	56
	HPV26	CCTGTGTTGATACCACCCGC	57
	HPV26	CAGCATCTGCATCCACTCCA	58
	HPV30	TGGCACCACTAGGAACACA	59
	HPV30	ATCTGCAACCACACAAACGT	60
25	HPV31	TGTCTGTTGTGCTGCAATT	61
	HPV31	AGATACCAACACGTAGTACCA	62
	HPV32	ATCTACGCCATGCAGAGGAA	63
	HPV32	ACTGTTGTGGATACTACCCG	64
	HPV33	TGGTAGATAACCACACGCAGT	65
30	HPV33	GCACACAAAGTAACTAGTGAC	66
	HPV34	CCACAAAGTACAACACTGCACCA	67
	HPV34	ACCTCAGACATGCAGAACAG	68
	HPV35	TGTCTGTTGTGCTGTG	69
	HPV35	AGGCATGGTAAGAATATGA	70
35	HPV39	ACTGTTGTGGACACTACCCG	71
	HPV39	TACCAAGGACGTGGAGGAGT	72
	HPV40	ATGTGCTGCCACACAGTCCC	73
	HPV40	TTTGCCTCATGGGGAGGAGT	74
	HPV42	GCCACTGCAACATCTGGTGA	75
	HPV42	ACTGTGGTTGATACTACCCG	76
	HPV44	GTGCTGCCACTACACAGTCC	77
	HPV44	CATGCGACATGTTGAGGAGT	78
40	HPV45	GTGGACACTACCCGCAGTAC	79
	HPV45	GTGCCAAGTACATATGACCC	80
	HPV47	TTACTCTCAGGGCAGGGACA	81
	HPV47	GTCACAGTTGAGACAAACAC	82
45	HPV51	GCACTGCCACTGCTGCGTT	83
50			
55			
60			

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TABLE 1-continued

Sequences		
Sequence Definition	Probe Sequence	SEQ ID NO:
HPV51	AGGCATGGGAAGAGTATGA	84
HPV52	ACCTTCGTCACTGGCAGGAA	85
HPV52	TGGATACCACACTCGTAGCACT	86
HPV53	ACTCTTCCGCAACCACACA	87
HPV53	TGTTGTGGATACCACCGAGGA	88
HPV54	GCTACAGCATCCACGCAGGA	89
HPV54	CAGTTGTAGATAACCACCGT	90
HPV56	ACCTTAGACATGTGGAGGAA	91
HPV56	CTGCTACAGAACAGTTAAGT	92
HPV58	GGTTGATACCACTCGTAGCA	93
HPV58	TGCACTGAAGTAACTAAGGA	94
HPV59	ACTACTCGCAGCACCAATCT	95
HPV59	ATGCCAGACATGTGGAGGAA	96
HPV61	CCGTTGTGGATACCACCGC	97
HPV61	TTGCGCCATACAGAGGAGTT	98
HPV62	TGTACCGCCTCCACTGCTGC	99
HPV62	TTTGCACACACGGAGGAAT	100
HPV66	ACACACGCCATGTAGAGGAA	101
HPV66	ACCAGAACGACCAACATGAC	102
HPVG7	ACACGTAGTACCAACATGAC	103
HPV67	ACCTTAGACATGTGGAGGAA	104
HPV68	TTGTGGATACACGCGCAGT	105
HPV68	CAGACTCTACTGTACCGACT	106
HPV69	ACCCGCAGTACCAACCTCAC	107
HPVG9	GCACAATCTGCATCTGCCAC	108
HPV70	TCTGCCTGCACCGAACCGC	109
HPV70	ACTGTGGTGGACACTACACG	110

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TABLE 1-continued

Sequences		
Sequence Definition	Probe Sequence	SEQ ID NO:
HPV71	ATGTCCATCTGTGCTACCAA	111
HPV71	ACAGTTGTGACACATCACGT	112
HPV72	ACTGCCACAGCGCCTCTGT	113
HPV72	ATCTTCGCCACACTGAGGAA	114
HPV73	GGTACACAGGCTAGTAGCTC	115
HPV73	CTACAACTGATGCCAACTCT	116
HPV74	ACCTCACAATGCCCTCTGC	117
HPV74	TGGATACCACACGCAGTACT	118
HPV82	GCACTGCTGTTACTCCATCT	119
HPV82	AGCAGTACATTAGGCATGGG	120
HPV82	GCACTGCTGCTACTCCATCA	121
HPV82	GCACAGACATTCACTCCAAC	122
HPV83	GCTGCTGCTACACAGGCTAA	123
HPV83	ACCTCCGCCACACAGAGGAA	124
HPV84	AGATACCACCCGCAGCACCA	125
HPV84	AGTGCTGCTACCAACACCGA	126
HPV85	ACACACGCCATGTAGAGGAA	127
HPV85	ACTGTGGTAGACACAACACG	128
HPV85	AGTGCCGCTACCCAGAAGGC	129
HPV86	TCGACACCACCCGCAGTACT	130
HPV87	TGCTGCCACTCAAACAACCA	131
HPV87	CGGTTGTTGATACTACTCGC	132
HPV89	GTGCTGCTCCAGTCTGGC	133
HPV89	ACCAACCGTAGTACCAACCT	134
HPV91	TGTGGATACAACTCGCAGCA	135
HPV91	GCATCCACTGAGTCTGTGCT	136

TABLE 2

History of Probe Development				
HPV TYPE OLIGO SEQUENCE 5' TO 3'	DNA SOURCE	BEAD #	COMMENTS	SEQ ID NO:
6 ACTACACGCAGTACCAACATGACATTATGT	PLASMID	50	low hybridization	137
CGTAACTACATCTTCCACATACACCAATT	PLASMID	50	no hybridization	138
CAACATGACATTATGTGCATCCGTAACTAC	PLASMID	50	no hybridization	139
CATGACATTATGTGCATCCGTAACTACATCTC	PLASMID	50	no hybridization	140
CATCTCCACATACACCAATTCTGATTATA	PLASMID	50	ok	1
TCCGTAACTACATCTCCACATACACCAAT	PLASMID	50	no hybridization	141
11 ACTATGTGCATCTGTGCTAAATCTGCTAC	PLASMID	13	good	2

TABLE 2-continued

History of Probe Development					
HPV TYPE OLIGO	SEQUENCE 5' TO 3'	DNA SOURCE	BEAD #	SEQ ID NO: COMMENTS	
13	AGCCACTACATCATCTCTTCAGACACATA TAACATGACTGTGTGCAGCCACTACATC GTGTGTGCAGCCACTACATCATCTCTTCA GTGTGTGCAGCCACTACATCATCTCTTCA	PLASMID PLASMID PLASMID PLASMID	34 34 34 34	no hybridization no hybridization no hybridization good	142 143 3 3
16	GCCATATCTACTTCAGAAAATCACATATAAA AAATATGTCATTATGTGCTGCCATATCTAC ATGTCATTATGTGCTGCCATATCTACTTCA GTCATTATGTGCTGCCATATCTACTTCAGA GCCATATCTACTTCAGAAAATCACATATAAA	PLASMID PLASMID PLASMID PLASMID PLASMID	98 98 98 98 98	no hybridization no hybridization cross hybridization with 62 cross hybridization	144 4 145 146 147
18	ATATGTGCTTCTACACAGTCTCTGTACCT AACATATGTGCTTCTACACAGTCTCTGT TCCTGACTCTGGCAATATGATGCTACCAA TATGTGCTTCTACACAGTCTCTGTACTCG TATGTGCTTCTACACAGTCTCTGTACTCG	PLASMID PLASMID PLASMID PLASMID PLASMID	15 15 15 15 15	good cross hybridization cross hybridization no hybridization no hybridization	5 148 149 150 150
26	CCTTACCATTAGTACATTATCTGCAGCATC AACCTTACCATTTAGTACATTATCTGCAGCA ACATTATCTGCAGCATTGCATCCACTCCA	PLASMID PLASMID PLASMID	5 5 5	good no hybridization good	6 151 152
30	ATCTGCAACCACACAAACGTTATCCACATA CCACACAAACGTTATCCACATATAATTCAA GACTATATCTGCAACACACAAACGTTATC ATCTGCAACACACAAACGTTATCCACATA	PLASMID PLASMID PLASMID PLASMID	53 40 40 40	good no hybridization no hybridization no hybridization	7 153 154 155
31	AAGTAGTAATTTAAAGAGTATTTAAGACA ATGCTGTTGTGCTGCAATTGCAAACAGT CAATATGCTGTTGTGCTGCAATTGCAA CAATATGCTGTTGTGCTGCAATTGCAA CAATATGCTGTTGTGCTGCAATTGCAA AACAGTGATACTACATTTAAAGTAGTAA TCTGTTGTGCTGCAATTGCAAACAGTGAT GCAATTGCAAACAGTGATACTACATTTAA	PLASMID PLASMID PLASMID PLASMID PLASMID PLASMID PLASMID PLASMID	55 55 55 25 25 25 25 55	no hybridization no hybridization low hybridization no hybridization no hybridization no hybridization no hybridization no hybridization	156 157 8 8 8 158 159 160
32	ACTGTAACAACTGAAGACACATACAAGTCT 7 CACCAACACCATATGCAATAGTAAGTTA	Test Sample 4330 Test Sample 3228	36 33	good take out not mucosal	9 161
33	TAGTGACAGTACATATAAAATGAAAAATT TAATATGACTTTATGCCACACAGTAACTAG GCACACAAGTAACTAGTGACAGTACATATA GTGACAGTACATATAAAATGAAAAATTAA	PLASMID PLASMID PLASMID PLASMID	58 30 58 30	cross hybridization ok no hybridization ok	162 10 163 164
34	TAGGTACACAATCCACAAAGTACAACGTGAC	PLASMID	37	no dna sample	165
35	TGTCTGTTCTGCTGTGCTCTAGTG GTGCTTCTAGTGACAGTACATATAAAAT AACCGTAGTACAAAATATGCTGTTGTT AAATATGCTGTTCTGCTGTTGCTTC TCTGCTGTTCTAGTGACAGTACATA	Test Sample 4498 Test Sample 4498 Test Sample 4498 Test Sample 4498 Test Sample 4498	30 30 77 77 77	no hybridization no hybridization no hybridization good no hybridization	166 167 168 11 169
39	CTTTACATTATCTACCTCTATAGAGTCTTC AGAGTCTCCATACCTCTACATGATGCC CCGTAGTACCAACTTACATTATCTACCTC CAACTTACATTATCTACCTCTATAGAGTC ATCTACCTCTATAGACTCTTCCATACCTTC CTACCTCTATAGAGTCTCCATACCTTCT	Test Sample 4317 Test Sample 4317 PLASMID PLASMID PLASMID Test Sample 4317	25 25 76 76 76 76	low hybridization low hybridization cross hybridization no hybridization good dna sample no good	170 171 172 173 12 174
40	GTCCCCCACACCAACCCCATATAAAACAG CTTATGTGCTGCCACACAGTCCCCCACCC ACCCCATATAAAACAGTAATTTCAGGAA ACAGTCCCCACACCAACCCCATATAAA	TEST SAMPLE 3343 TEST SAMPLE 3343 TEST SAMPLE 3343 TEST SAMPLE 3343	38 38 38 38	cross hybridization ok cross hybridization cross hybridization	175 13 176 177
42	TCTGGTGATACTACACAGTGTCTAA ACATCTGGTGATACTACACAGTGTCTAA CACTGCAACATCTGGTGATACTACAGC	test sample none Test Sample 3398	42 42 42	multiple infection cross hybridization dna sample not good	14 178 179
43	AAACTTAACGTTATGTGCCCTACTGACCC TGACCCACTGTGCCAGTACATATGACAA TGCAAAGTTAAGGAATACCTGCGGCA	none none none	64 64 64	waiting cross hybridization cross hybridization	15 180 181
44	GCCACTACACAGTCCCCCTCCGTCTACATAT GACAATATGTCGTGCCACTACACAGTCCCC AAACATGACAATATGTCGTGCCACTACACA AATATGTCGTGCCACTACACAGTCCCC	PLASMID PLASMID PLASMID PLASMID	3 10 10 10	no hybridization ok good cross hybridization	182 183 16 184

TABLE 2-continued

TABLE 2-continued

History of Probe Development					
HPV TYPE	OLIGO SEQUENCE 5' TO 3'	DNA SOURCE	BEAD #	COMMENTS	SEQ ID NO:
90	CACACAAACACCCTCTGACACATACAAGGC	test sample 4015	83	cross hybridization	227
91	TAACTAACCTTGTGCTCATCCACTGAGTC	test sample 50211	63	hybridizes with 89	45
91	CTACCTACTACATATGACAACACAAAGTTC	find new dna	63	no hybridization	228
91	ATCCACTGAGTCTGTGCTACCTACTACATA	find new dna	63	no hybridization	229
97	TTAACACTGTGTGCTTCTACACAAAATGG	PLASMID		Fair	230
97	TCTACACAAAATGGCGTAGCTACCACATAT	PLASMID		good	46

TABLE 3

Probe Sequences
Final list of probes for the detection
of 46 HPV types with the Luminex
Microsphere technology.

SEQ ID NO:	HPV TYPE	OLIGO SEQUENCE 5' TO 3'
1	6	CATCTTCCACATACACCAATTCTGATTATA
2	11	ACTATGTGCATCTGTGCTAAATCTGCTAC
3	13	GTGTGTCGCCACTACATCATCTCTTCA
4	16	AAATATGTCAATTATGTGCTGCCATATCTAC
5	18	ATATGTGCTCTACACAGTCTCCTGTACCT
6	26	CCTTACCAATTAGTACATTATCTGCAGCATC
7	30	ATCTGCAACCACACAAACGTTATCCACATA
8	31	CAATATGTCTGTTGTGCTGCAATTGCAA
9	32	ACTGTAACAACGTGAAGACACATACAAGTCT
10	33	TAATATGACTTTATGCACACAAGTAACAG
11	35	AAATATGTCTGTGTTCTGCTGTCTTC
12	39	ATCTACCTCTATAGAGTCTCCATACCTTC
13	40	CTTATGTGCTGCCACACAGTCCCCCACACC
14	42	TCTGGTGATAACATACAGCTGCTAATTT
15	43	AAACTAACGTTATGTGCTCTACTGACCC
16	44	AAACATGACAATATGTGCTGCCACTACACA
17	45	TAATTTAACATTATGTGCTCTACACAAA
18	51	GCCACTGCTGCGGTTCCCCACATTTACTC
19	52	GACTTTATGTGCTGAGGTTAAAAGGAAAG
20	53	CGCAACCACACAGTCTATGTCTACATATAA
21	54	ACAGCATCCACGCAGGATAGCTTAAATAT
22	56	CATGACTATTAGTACTGCTACCAGAACAGT
23	58	ATGACATTATGCACTGAAGTAACAGGAA
24	59	CTTCTGTGCTGCTTCTACTACTCTTCT
25	61	CATTTGACTGCTACATCCCCCCTGTATC

TABLE 3-continued

Probe Sequences
Final list of probes for the detection
of 46 HPV types with the Luminex
Microsphere technology.

SEQ ID NO:	HPV TYPE	OLIGO SEQUENCE 5' TO 3'
26	62	ACCGCCTCCACTGCTGCAGCAGAATACACG
30	27	GACTATTAAATGCAGCTAAAGCACATTAAAC
35	28	TCTGAGGAAAATCAGAGGCTACATACAAA
30	29	ATTGTCCACTACTACAGACTCTACTGTACC
35	30	ACTGTATCTGCACAATCTGCATCTGCCACT
31	31	GTCTGCCTGCACCGAAACGGCCATACCTGC
32	32	ACCAAAACTGTGAGTCTACATATAAAGCC
40	33	CAGCTTCTAAATTCGTGAGTATCTCGCC
34	34	TAGGTACACAGGCTAGTAGCTCTACTACAA
45	35	TAACATGACTGTGCTGCTCCACCTCACAA
36	36	TACTATTGACAGCTACATCTGCTGTC
37	37	GCTGTTACTCCATCTGTTGCACAAACATT
50	38	CAGCTGCTGCTACACAGGCTAATGAATACA
39	39	GCTACCAACACCGAATCAGAATATAAACCT
40	40	TGCAACTACTAACCTCAGTCCATCTATATA
55	41	TAATTTACTATTAGTGCCTGCTACCCAGAA
42	42	CAATTTACTATTAGTGCCTGCTACCAAC
43	43	GCTTCCCAGTCTGGCACAGAATAC
60	44	CACCAATATGACTATTGTGCCACACAAAC
45	45	TAACCTAACCTGTGCTCATCCACTGAGTC
65	46	TCTACACAAAATGGCGTAGCTACCACATAT

TABLE 4

Determination of specificity and sensitivity of the 46 probes (columns) for hybridization with the cognate HPV DNA amplified from cloned controls for each HPV type (rows).

Sample	HPV 6	HPV 11	HPV 13	HPV 16	HPV 18	HPV 26	HPV 30
HPV 6	2004	0	2	0	0	0	0
HPV 11	17	1445	-8	-7	57	5	-5
HPV 13	1	3	1061	1	-17	-12	8
HPV 16	0	0	0	3378	0	0	0
HPV 18	0	0	0	15	2802	0	0
HPV 26	0	5	0	0	0	1961	0
HPV 30	0	0	0	0	0	0	5008
HPV 31	0	0	0	0	0	0	0
HPV 32	0	0	0	0	0	0	0
HPV 33	3	0	0	6	0	0	0
HPV 35	0	0	0	0	0	0	0
HPV 39	0	0	0	0	0	0	0
HPV 40	0	0	0	0	0	0	0
HPV 42	0	0	0	0	0	0	0
HPV 43	0	0	0	0	0	0	0
HPV 44	-9	4	7	-16	15	5	2
HPV 45	0	0	0	0	0	0	0
HPV 51	0	0	0	0	0	0	0
HPV 52	5	14	3	-5	16	-4	-13
HPV 53	0	0	0	0	0	0	0
HPV 54	0	0	0	0	0	0	0
Sample	HPV 6	HPV 11	HPV 13	HPV 16	HPV 18	HPV 26	HPV 30
HPV 54	0	0	0	0	0	0	0
HPV 56	0	0	0	0	0	0	0
HPV 58	0	0	0	0	0	0	0
HPV 59	0	0	0	0	0	0	0
HPV 61	0	0	0	0	0	0	0
HPV 62	-4	3	3	4	-16	-13	-17
HPV 66	0	0	0	0	0	0	0
HPV 67	0	0	0	0	0	0	0
HPV 68	8	25	-7	1	-2	-15	23
HPV 69	8	-3	1	-15	7	-13	-13
HPV 70	0	0	0	0	0	0	0
HPV 71	2	-9	17	9	6	-77	-77
HPV 72	0	0	0	0	0	0	0
HPV 73	5	34	15	2	12	18	18
HPV 74	5	7	-1	3	-11	18	18
HPV 81	14	-5	-18	-18	-6	7	-13
HPV 82	0	0	0	0	0	0	0
HPV 83	0	-11	-4	1	-19	-6	-6
HPV 84	-10	-9	-6	10	-2	-2	-2
HPV 85	0	0	0	0	0	0	0
HPV 86	7	6	13	32	-1	7	7
HPV 87	-9	5	-18	-6	13	-13	-13
HPV 89	-5	10	5	4	3	-4	-4
HPV 90	15	-11	9	13	2	-2	-2
HPV 91	19	10	22	14	-2	1	1
HPV 97	-8	-5	-3	-1	12	7	7
Sample	HPV 31	HPV 32	HPV 33	HPV 35	HPV 39	HPV 40	HPV 42
HPV 6	0	0	5	0	0	0	0
HPV 11	-23	1	-91	10	0	-13	-3
HPV 13	-45	-2	-106	-13	-6	13	-11
HPV 16	0	9	9	7	0	0	0
HPV 18	1	0	0	0	0	0	0
HPV 26	0	9	0	0	2	0	0
HPV 30	0	0	17	0	0	0	0
HPV 31	1168	0	27	0	11	0	0
HPV 32	0	2601	5	0	0	0	0
HPV 33	0	2	1083	0	0	0	2
HPV 35	0	0	13	2348	1	0	0
HPV 39	0	0	0	0	3554	0	0
HPV 40	0	0	0	0	0	4154	0
HPV 42	0	0	0	0	0	0	3689
HPV 43	0	0	0	0	0	0	0
HPV 44	-23	-4	12	0	-3	1	-7
HPV 45	0	0	0	0	0	0	0
HPV 51	0	0	0	2	0	0	0
HPV 52	4	11	-24	23	2	13	-13

TABLE 4-continued

Determination of specificity and sensitivity of the 46 probes (columns) for hybridization with the cognate HPV DNA amplified from cloned controls for each HPV type (rows).

HPV 53	0	0	0	0	0	0	0
HPV 54	0	0	0	0	0	0	0
Sample	HPV 31	HPV 32	HPV 33	HPV 35	HPV 39	HPV 40	HPV 42
HPV 54	0	0	0	0	0	0	0
HPV 56	0	0	0	0	0	0	0
HPV 58	0	0	0	0	0	0	0
HPV 59	0	0	0	0	0	0	0
HPV 61	0	0	0	0	0	0	0
HPV 62	-10	-41	-6	7	0	-3	-4
HPV 66	0	0	0	0	0	0	0
HPV 67	0	0	0	0	0	0	0
Hpv 68	4	1	-62	-5	3	-11	3
HPV 69	-34	25	-56	0	4	3	2
HPV 70	0	0	0	0	0	0	0
HPV 71	-7	-58	-7	-9	13	12	-8
HPV 72	0	0	0	14	0	0	0
HPV 73	32	12	23	22	18	2	22
HPV 74	-4	-1	-40	-4	4	6	20
HPV 81	30	9	59	18	-3	1	34
HPV 82	0	0	0	0	0	0	0
HPV 83	-7	9	-46	7	-17	-3	14
HPV 84	-12	7	-4	9	18	7	-4
HPV 85	0	0	0	0	0	0	0
HPV 86	-15	23	-10	16	-3	1	12
HPV 87	-10	-7	-46	-13	7	3	6
HPV 89	-18	15	-5	-13	-5	2	-2
HPV 90	8	10	-6	33	0	32	43
HPV 91	17	3	8	-3	22	13	9
HPV 97	-2	4	-30	4	-4	-7	-2
Sample	HPV 43	HPV 44	HPV 45	HPV 51	HPV 52	HPV 53	HPV 54
HPV 6	0	0	0	0	0	0	0
HPV 11	-15	-9	-19	15	-5	-7	-6
HPV 13	-6	-12	-14	-30	5	8	-11
HPV 16	0	5	0	0	0	0	0
HPV 18	0	8	5	0	0	0	0
HPV 26	9	0	0	18	0	0	0
HPV 30	0	6	14	0	0	0	6
HPV 31	0	0	0	0	0	0	0
HPV 32	0	0	0	0	0	0	0
HPV 33	4	0	0	8	0	0	0
HPV 35	0	3	0	0	0	0	0
HPV 39	0	0	0	0	0	0	0
HPV 40	0	0	0	0	0	0	0
HPV 42	0	0	0	0	0	0	0
HPV 43	3364	0	0	0	0	0	0
HPV 44	27	2068	-8	-31	0	14	2
HPV 45	0	0	1922	0	0	0	0
HPV 51	0	0	0	2884	0	0	0
HPV 52	0	6	22	-20	2429	-10	-5
HPV 53	0	0	0	0	0	2162	0
HPV 54	0	0	0	0	0	0	2755
Sample	HPV 43	HPV 44	HPV 45	HPV 51	HPV 52	HPV 53	HPV 54
HPV 54	0	0	0	0	0	0	2755
HPV 56	0	0	0	0	0	0	0
HPV 58	0	0	0	0	0	0	0
HPV 59	0	0	0	0	0	0	0
HPV 61	0	0	0	0	0	0	0
HPV 62	12	0	-6	-5	1	-3	-19
HPV 66	0	0	0	0	0	0	0
HPV 67	0	0	0	0	0	0	0
Hpv 68	8	-12	-3	-14	-7	8	6
HPV 69	8	9	3	-1	2	0	27
HPV 70	0	0	0	0	0	0	0
HPV 71	-15	-10	11	-1	-10	1	-9
HPV 72	0	0	0	0	0	0	0
HPV 73	35	24	31	20	17	0	17
HPV 74	4	-3	-13	3	-3	5	-11

TABLE 4-continued

Determination of specificity and sensitivity of the 46 probes (columns) for hybridization with the cognate HPV DNA amplified from cloned controls for each HPV type (rows).

	HPV 81	33	36	-12	7	-8	13	-10	
Sample	HPV 56	HPV 58	HPV 59	HPV 61	HPV 62	HPV 66	HPV 67	HPV 68	HPV 69
HPV 6	0	0	0	5	0	0	0	0	0
HPV 11	-3	14	2	-13	6	-4	5	-1	-10
HPV 13	10	-6	-15	-3	-11	-10	-19	16	-12
HPV 16	0	0	0	6	0	0	0	0	0
HPV 18	0	0	0	0	0	0	0	0	0
HPV 26	0	0	0	0	0	0	0	0	0
HPV 30	4	0	0	0	0	0	14	0	14
HPV 31	0	0	0	0	0	0	0	0	0
HPV 32	0	0	0	0	18	0	16	18	0
HPV 33	0	0	0	0	0	0	4	0	15
HPV 35	7	0	0	0	0	0	23	6	0
HPV 39	0	0	0	5	0	0	0	0	0
HPV 40	0	0	0	0	0	0	0	0	0
HPV 42	0	0	0	0	0	0	0	0	0
HPV 43	0	0	0	0	0	0	0	0	0
HPV 44	-13	-4	5	-8	-15	-12	-3	20	-9
HPV 45	0	0	0	0	0	0	0	0	0
HPV 51	0	0	0	0	0	0	0	0	0
HPV 52	-4	16	-5	-6	0	-5	-31	-3	5
HPV 53	0	0	0	0	0	0	0	0	0
HPV 54	0	0	0	0	0	0	0	0	0
Sample	HPV 56	HPV 58	HPV 59	HPV 61	HPV 62	HPV 66	HPV 67	HPV 68	HPV 69
HPV 56	1638	0	0	0	0	0	0	0	0
HPV 58	0	1874	0	0	0	0	0	0	0
HPV 59	0	0	3405	0	0	0	0	0	0
HPV 61	0	0	0	3831	0	0	0	0	0
HPV 62	-11	-13	-6	2	2298	-2	-12	10	14
HPV 66	0	0	0	0	0	1258	0	0	0
HPV 67	0	0	0	0	0	0	1115	0	0
HPV 68	6	-11	-18	15	-6	8	12	957	5
HPV 69	-10	7	-12	-1	-4	8	-8	21	1909
HPV 70	0	0	0	0	0	0	0	0	0
HPV 71-3	1		-1	-2	-17	20	-11	-19	-2
HPV 72	0	0	0	0	0	0	0	0	0
HPV 73	11	0	0	25	38	4	37	23	39
HPV 74	-10	-3	5	20	-4	3	15	8	1
HPV 81	-9	0	-27	3	-10	-10	47	47	4
HPV 82	0	0	0	0	0	0	0	0	0
HPV 83	15	-3	-9	9	-15	11	-6	8	2
HPV 84	-12	0	3	-2	1	-6	-16	17	-9
HPV 85	0	0	0	0	0	0	6	0	0
HPV 86	15	-2	-3	1	6	12	5	32	14
HPV 87	-7	-13	-8	-8	16	-13	7	31	3
HPV 89	-3	-3	-1	-5	4	15	7	7	-14
HPV 90	1	-6	2	11	6	5	-24	40	-10
HPV 91	11	3	-3	13	9	5	32	16	15
HPV 97	-3	-6	5	-16	-4	-9	-8	3	7
Sample	HPV 70	HPV 71	HPV 72	HPV 73	HPV 74	HPV 81	HPV 82	HPV 83	
HPV 6	1	2	0	0	0	0	2	0	
HPV 11	-10	143	-10	2	-6	-9	-18	-6	
HPV 13	0	-11	254	4	26	5	-12	-15	
HPV 16	0	0	15	0	4	0	0	0	
HPV 18	0	3	12	0	0	0	0	0	
HPV 26	0	0	21	0	0	0	1	0	
HPV 30	0	8	0	0	0	0	0	0	

TABLE 4-continued

Determination of specificity and sensitivity of the 46 probes (columns) for hybridization with the cognate HPV DNA amplified from cloned controls for each HPV type (rows).

TABLE 4-continued

Determination of specificity and sensitivity of the 46 probes (columns) for hybridization with the cognate HPV DNA amplified from cloned controls for each HPV type (rows).

HPV 59	0	0	0	0	0	0	0	-22
HPV 61	0	0	0	0	0	0	0	-21
HPV 62	21	-15	-12	-4	2	-5	29	-20
HPV 66	0	0	0	0	0	0	0	-7
HPV 67	0	0	0	0	0	0	0	-5
HPV 68	4	-11	-4	-5	-2	2	0	-11
HPV 69	8	4	-6	-1	11	-8	6	-28
HPV 70	0	0	0	0	11	0	0	1
HPV 71	10	-13	-4	-19	4	-18	-5	10
HPV 72	0	0	0	0	0	0	0	-13
HPV 73	14	15	24	9	4	36	27	-12
HPV 74	-7	-7	7	3	12	0	19	1
HPV 81	-20	-7	4	5	-3	7	-9	-18
HPV 82	0	0	0	0	0	0	0	-14
HPV 83	19	-17	6	6	-1	15	8	-7
HPV 84	2194	19	0	15	-13	-5	-15	-16
HPV 85	0	4605	0	0	0	0	0	-17
HPV 86	18	5	1720	35	12	13	2	-10
HPV 87	-3	-1	8	1828	12	7	1	0
HPV 89	9	12	-13	-10	3998	-27	1	-19
HPV 90	13	7	15	16	23	3613	5	-13
HPV 91	-8	6	14	21	24	31	2264	-16
HPV 97	6	2	-20	-3	-3	-7	-11	1267

TABLE 5

Comparison of NML Luminex with direct sequencing for detection of any HPV type
Direct sequencing

NML Luminex	Negative	Positive	Totals
Positive	14	429	442
Negative	348	5	353
Totals	361	434	795

TABLE 6-continued

Distribution of HPV types as detected by NML Luminex and direct sequencing

HPV type	n	NML LUMINEX		Direct Sequencing	
		% of types	% of positive samples	% of types	% of positive samples
58	28	4.9%	3.5%	25	3.2%
59	11	1.9%	1.4%	5	0.6%
61	7	1.2%	0.9%	1	0.1%
62	26	4.5%	3.3%	17	2.1%
66	39	6.8%	4.9%	30	3.8%
67	7	1.2%	0.9%	8	1.0%
68	2	0.3%	0.3%	4	0.5%
69	3	0.5%	0.4%	2	0.3%
70	11	1.9%	1.4%	10	1.3%

TABLE 6

Distribution of HPV types as detected by NML Luminex and direct sequencing

HPV type	n	NML LUMINEX		Direct Sequencing	
		% of types	% of positive samples	% of types	% of positive samples
6	43	7.5%	5.4%	39	4.9%
11	12	2.1%	1.5%	11	1.4%
13	0	0.0%	0.0%	0	0.0%
16	87	15.1%	10.9%	68	8.6%
18	26	4.5%	3.3%	15	1.9%
26	0	0.0%	0.0%	0	0.0%
30	2	0.3%	0.3%	0	0.0%
31	29	5.0%	3.6%	29	3.7%
32	3	0.5%	0.4%	2	0.3%
33	11	1.9%	1.4%	14	1.8%
35	8	1.4%	1.0%	2	0.3%
39	22	3.8%	2.8%	16	2.0%
40	9	1.6%	1.1%	5	0.6%
42	13	2.3%	1.6%	5	0.6%
43	0	0.0%	0.0%	1	0.1%
44	3	0.5%	0.4%	1	0.1%
45	12	2.1%	1.5%	10	1.3%
51	16	2.8%	2.0%	9	1.1%
52	33	5.7%	4.2%	17	2.1%
53	25	4.3%	3.1%	12	1.5%
54	11	1.9%	1.4%	8	1.0%
56	10	1.7%	1.3%	4	0.5%

HPV type	n	NML LUMINEX		Direct Sequencing	
		% of types	% of positive samples	% of types	% of positive samples
58	28	4.9%	3.5%	25	3.2%
59	11	1.9%	1.4%	5	0.6%
61	7	1.2%	0.9%	1	0.1%
62	26	4.5%	3.3%	17	2.1%
66	39	6.8%	4.9%	30	3.8%
67	7	1.2%	0.9%	8	1.0%
68	2	0.3%	0.3%	4	0.5%
69	3	0.5%	0.4%	2	0.3%
70	11	1.9%	1.4%	10	1.3%
71	3	0.5%	0.4%	0	0.0%
72	5	0.9%	0.6%	4	0.5%
73	5	0.9%	0.6%	3	0.4%
74	1	0.2%	0.1%	0	0.0%
81	8	1.4%	1.0%	6	0.8%
82	7	1.2%	0.9%	5	0.6%
83	11	1.9%	1.4%	5	0.6%
84	12	2.1%	1.5%	1	0.1%
85	2	0.3%	0.3%	2	0.3%
86	1	0.2%	0.1%	1	0.1%
87	2	0.3%	0.3%	3	0.4%
88	8	1.4%	1.0%	4	0.5%
89	3	0.5%	0.4%	1	0.1%
90	3	0.5%	0.4%	0	0.0%
91	1	0.2%	0.1%	0	0.0%
102	N/A	N/A	N/A	2	0.3%

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TABLE 7

Comparison of NML Luminex vs Roche linear array for detection of samples positive for any HPV type.

Roche Linear Array			
NML Luminex	Negative	Positive	Totals
Positive	46	394	440
Negative	424	16	440
Totals	470	410	880

TABLE 8

Comparison between NML Luminex and Roche Linear array in the ability to detect multiple HPV infections.

	NML Luminex	Roche Linear Array
Positive for any type	435	405
Total HPV types detected	917	1111
Single infections	200	156
Multiple infections	235	249
2 types	122	87
3 types	49	59
4+ types	64	103

TABLE 9

Comparison of HPV type distribution as detected by NML Luminex and the Roche Linear Array method.

	NML Luminex	Roche Linear Array		
n	%	n	%	
HPV06	48	5.2%	49	4.4%
HPV11	39	4.3%	45	4.1%
HPV13*	0	0.0%		
HPV16	136	14.8%	137	12.3%
HPV18	47	5.1%	41	3.7%
HPV26	0	0.0%	3	0.3%
HPV30*	2	0.2%		
HPV31	31	3.4%	48	4.3%
HPV32*	11	1.2%		
HPV33	15	1.6%	16	1.4%
HPV35	30	3.3%	23	2.1%
HPV39	27	2.9%	39	3.5%
HPV40	10	1.1%	8	0.7%
HPV42	30	3.3%	38	3.4%
HPV43*	3	0.3%		
HPV44	11	1.2%	16	1.4%
HPV45	34	3.7%	30	2.7%
HPV51	31	3.4%	57	5.1%
HPV52**	26	2.8%	74	6.7%
HPV53**	25	2.7%	53	4.8%
HPV54	14	1.5%	21	1.9%
HPV56	34	3.7%	29	2.6%
HPV58	27	2.9%	30	2.7%
HPV59	31	3.4%	44	4.0%
HPV61**	10	1.1%	27	2.4%
HPV62	14	1.5%	30	2.7%
HPV66	38	4.1%	38	3.4%
HPV67**	19	2.1%	6	0.5%
HPV68	13	1.4%	21	1.9%
HPV69	9	1.0%	9	0.8%
HPV70	30	3.3%	29	2.6%
HPV71	1	0.1%	1	0.1%
HPV72	7	0.8%	12	1.1%
HPV73**	6	0.7%	18	1.6%
HPV74*	12	1.3%		
HPV81	12	1.3%	12	1.1%
HPV82	4	0.4%	11	1.0%
HPV83	12	1.3%	18	1.6%

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TABLE 9-continued

Comparison of HPV type distribution as detected by NML Luminex and the Roche Linear Array method.

5	NML Luminex		Roche Linear Array		
	n	%	n	%	
10	HPV84**	18	2.0%	39	3.5%
	HPV85*	5	0.5%		
	HPV86*	11	1.2%		
	HPV87	4	0.4%		
	HPV89**	18	2.0%	38	3.4%
	HPV90*	12	1.3%		
	HPV91*	0	0.0%		

*Type not detected by the Roche Linear Array

**Statistically significant difference ($p < 0.05$)

TABLE 10

Comparison of HPV type distribution as detected by NML Luminex and the Roche Linear Array method when multiple infections with 4 or more types are excluded.

20	NML Luminex		Roche Linear Array		
	Strain	n	%	n	%
30	HPV06	26	4.9%	23	4.3%
	HPV11	26	4.9%	30	5.6%
	HPV13*	0	0		
	HPV16	86	16.1%	90	16.9%
	HPV18	24	4.5%	17	3.2%
	HPV26	0	0%	0	0%
	HPV30*	1	0.2%		
	HPV31	24	4.5%	25	4.7%
	HPV32*	5	0.9%	0	0.0%
	HPV33	7	1.3%	5	0.9%
	HPV35	14	2.6%	8	1.5%
	HPV39	15	2.8%	16	3.0%
	HPV40	8	1.5%	4	0.7%
	HPV42	21	3.9%	21	3.9%
	HPV43*	2	0.4%		
	HPV44	9	1.7%	5	0.9%
	HPV45	12	2.2%	8	1.5%
	HPV51	23	4.3%	29	5.4%
	HPV52**	15	2.8%	34	6.4%
	HPV53	19	3.6%	25	4.7%
	HPV54	10	1.9%	12	2.2%
	HPV56	20	3.7%	11	2.1%
	HPV58	14	2.6%	11	2.1%
	HPV59	11	2.1%	16	3.0%
	HPV61	6	1.1%	14	2.6%
	HPV62	10	1.9%	16	3.0%
	HPV66	21	3.9%	19	3.6%
	HPV67**	10	1.9%	2	0.4%
	HPV68	9	1.7%	8	1.5%
	HPV69	5	0.9%	5	0.9%
	HPV70	15	2.8%	12	2.2%
	HPV71	0	0%		
	HPV72	4	0.7%	6	1.1%
	HPV73	4	0.7%	9	1.7%
	HPV74*	7	1.3%	0	0.0%
	HPV81	4	0.7%	3	0.6%
	HPV82	2	0.4%	4	0.7%
	HPV83	8	1.5%	10	1.9%
	HPV84	10	1.9%	17	3.2%
	HPV85*	2	0.4%		
	HPV86*	5	0.9%		
	HPV87	1	0.2%		
	HPV89	11	2.1%	18	3.4%
	HPV90*	9	1.7%		
	HPV91*	0	0%		

SEQUENCE LISTING

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<400> SEQUENCE: 2

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gttgtgtcag ccactacatc atctctttca

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<212> TYPE: DNA	
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<400> SEQUENCE: 36
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<210> SEQ ID NO 37
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<400> SEQUENCE: 37
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<400> SEQUENCE: 38
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<210> SEQ ID NO 39
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<400> SEQUENCE: 39
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<213> ORGANISM: Human papillomavirus

<400> SEQUENCE: 40

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<400> SEQUENCE: 41

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<213> ORGANISM: Human papillomavirus

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24

<210> SEQ ID NO 44

<211> LENGTH: 30

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<213> ORGANISM: Human papillomavirus

<400> SEQUENCE: 44

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<211> LENGTH: 30

<212> TYPE: DNA

<213> ORGANISM: Human papillomavirus

<400> SEQUENCE: 45

taacttaacc ttgtgtcat ccactgagtc

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<210> SEQ ID NO 46

<211> LENGTH: 30

<212> TYPE: DNA

<213> ORGANISM: Human papillomavirus

<400> SEQUENCE: 46

tctacacaaa atggcgtagc taccacatat

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<210> SEQ ID NO 47

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Human papillomavirus type 6

<400> SEQUENCE: 47

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<210> SEQ ID NO 48

<211> LENGTH: 20

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<212> TYPE: DNA
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<400> SEQUENCE: 48

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<210> SEQ ID NO 49
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<400> SEQUENCE: 49

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<210> SEQ ID NO 50
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<400> SEQUENCE: 50

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<210> SEQ ID NO 51
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<400> SEQUENCE: 51

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<400> SEQUENCE: 53

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<400> SEQUENCE: 54

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<210> SEQ ID NO 55
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<210> SEQ ID NO 56

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<210> SEQ ID NO 57
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<400> SEQUENCE: 57
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<210> SEQ ID NO 58
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<400> SEQUENCE: 58
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<400> SEQUENCE: 59
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<400> SEQUENCE: 60
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<210> SEQ ID NO 61
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<400> SEQUENCE: 61
tgtctgtttg tgctgcaatt                                20

<210> SEQ ID NO 62
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<213> ORGANISM: Human papillomavirus type 31

<400> SEQUENCE: 62
agataccaca cgttagttacca                                20

<210> SEQ ID NO 63
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<213> ORGANISM: Human papillomavirus type 32

<400> SEQUENCE: 63
atctacgcca tgcagaggaa                                20

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<210> SEQ ID NO 64
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<213> ORGANISM: Human papillomavirus type 32

<400> SEQUENCE: 64

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<210> SEQ ID NO 65
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<400> SEQUENCE: 65

tggtagatac cactcgagt

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<210> SEQ ID NO 66
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<400> SEQUENCE: 66

gcacacaagt aactagtgac

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<210> SEQ ID NO 67
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<400> SEQUENCE: 67

ccacaagtac aactgcacca

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<210> SEQ ID NO 68
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<212> TYPE: DNA
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<400> SEQUENCE: 68

acctcagaca tgcagaagag

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<210> SEQ ID NO 69
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<213> ORGANISM: Human papillomavirus type 35

<400> SEQUENCE: 69

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<210> SEQ ID NO 70
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<212> TYPE: DNA
<213> ORGANISM: Human papillomavirus type 35

<400> SEQUENCE: 70

aggcatggtg aagaatatga

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<210> SEQ ID NO 71
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Human papillomavirus type 39

<400> SEQUENCE: 71

actgttgtgg acactacccg

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<210> SEQ ID NO 72
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<400> SEQUENCE: 72

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<210> SEQ ID NO 73
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Human papillomavirus type 40

<400> SEQUENCE: 73

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<210> SEQ ID NO 74
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<400> SEQUENCE: 74

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<213> ORGANISM: Human papillomavirus type 42

<400> SEQUENCE: 75

gccactgcaa catctggta

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<210> SEQ ID NO 76
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<400> SEQUENCE: 76

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<210> SEQ ID NO 77
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<400> SEQUENCE: 77

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<400> SEQUENCE: 78

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<400> SEQUENCE: 79

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<400> SEQUENCE: 80

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<400> SEQUENCE: 81

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<210> SEQ ID NO 82
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gtcacagttg tagacaacac

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<210> SEQ ID NO 83
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<400> SEQUENCE: 83

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aggcatgggg aagagtatga

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<210> SEQ ID NO 85
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<400> SEQUENCE: 85

accttcgtca tggcgaggaa

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<210> SEQ ID NO 86
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<400> SEQUENCE: 86

tggataaccac tcgttagcact

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<211> LENGTH: 20
<212> TYPE: DNA
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<400> SEQUENCE: 87

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<210> SEQ ID NO 90
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<210> SEQ ID NO 92
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<210> SEQ ID NO 94
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<212> TYPE: DNA
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<400> SEQUENCE: 94

tgcactgaag taactaagga	20
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<210> SEQ ID NO 95
<211> LENGTH: 20
<212> TYPE: DNA
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<400> SEQUENCE: 95

actactcgca gcaccaatct	20
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<210> SEQ ID NO 96
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<212> TYPE: DNA
<213> ORGANISM: Human papillomavirus type 59
<400> SEQUENCE: 96

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<210> SEQ ID NO 97
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ccgttgtgga taccacccgc	20
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<210> SEQ ID NO 98
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<400> SEQUENCE: 98

ttgcgccata cagaggagt	20
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<210> SEQ ID NO 99
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<212> TYPE: DNA
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<210> SEQ ID NO 104

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<212> TYPE: DNA

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<210> SEQ ID NO 105

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Human papillomavirus type 68

<400> SEQUENCE: 105

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<210> SEQ ID NO 106

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<212> TYPE: DNA

<213> ORGANISM: Human papillomavirus type 68

<400> SEQUENCE: 106

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<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Human papillomavirus type 69

<400> SEQUENCE: 107

acccgcagta ccaacctcac

20

<210> SEQ ID NO 108

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Human papillomavirus type 69

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<400> SEQUENCE: 113

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acctcacaat cgccttctgc

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<400> SEQUENCE: 119

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<400> SEQUENCE: 124

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<400> SEQUENCE: 125

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<400> SEQUENCE: 126

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<213> ORGANISM: Human papillomavirus

<400> SEQUENCE: 133

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<213> ORGANISM: Human papillomavirus

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<210> SEQ ID NO 135

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<400> SEQUENCE: 135

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<400> SEQUENCE: 136

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<400> SEQUENCE: 137

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<213> ORGANISM: Human papillomavirus type 6

<400> SEQUENCE: 138

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<210> SEQ ID NO 139
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<212> TYPE: DNA
<213> ORGANISM: Human papillomavirus type 6

<400> SEQUENCE: 139

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<210> SEQ ID NO 140
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<212> TYPE: DNA
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<400> SEQUENCE: 140

catgacatta tgtgcatccg taactacatc ttc                                33

<210> SEQ ID NO 141
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<400> SEQUENCE: 142

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<400> SEQUENCE: 157
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<210> SEQ ID NO 162
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<210> SEQ ID NO 163
<211> LENGTH: 30
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gcacacaagt aactagtgac agtacatata 30

<210> SEQ ID NO 164
<211> LENGTH: 30
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gtgacagttac atataaaaaat gaaaatttta 30

<210> SEQ ID NO 165
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<400> SEQUENCE: 165

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<210> SEQ ID NO 166
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<400> SEQUENCE: 166

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<210> SEQ ID NO 168
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<400> SEQUENCE: 169

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<210> SEQ ID NO 170
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<212> TYPE: DNA
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<400> SEQUENCE: 170

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<210> SEQ ID NO 172
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<210> SEQ ID NO 173
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<212> TYPE: DNA
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<400> SEQUENCE: 173

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<210> SEQ ID NO 174
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<212> TYPE: DNA
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<400> SEQUENCE: 174

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<210> SEQ ID NO 176
<211> LENGTH: 30
<212> TYPE: DNA
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<400> SEQUENCE: 176

accccatata ataacagtaa tttcaaggaa 30

<210> SEQ ID NO 177
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<212> TYPE: DNA
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acagtcccc acaccaaccc catataataa 30

<210> SEQ ID NO 178
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<212> TYPE: DNA
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acatctggtg atacatatac agctgcta 30

<210> SEQ ID NO 179
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Human papillomavirus type 42
<400> SEQUENCE: 179

cactgcaaca tctggtgata catatacago 30

<210> SEQ ID NO 180
<211> LENGTH: 30
<212> TYPE: DNA
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tgaccctact gtgcccgatca catatgacaa 30

<210> SEQ ID NO 181
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<213> ORGANISM: Human papillomavirus type 43
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<210> SEQ ID NO 182
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<400> SEQUENCE: 183	
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<212> TYPE: DNA	
<213> ORGANISM: Human papillomavirus type 45	
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<210> SEQ ID NO 186	
<211> LENGTH: 30	
<212> TYPE: DNA	
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<400> SEQUENCE: 186	
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<210> SEQ ID NO 187	
<211> LENGTH: 30	
<212> TYPE: DNA	
<213> ORGANISM: Human papillomavirus type 51	
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<212> TYPE: DNA	
<213> ORGANISM: Human papillomavirus type 51	
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<210> SEQ ID NO 189	
<211> LENGTH: 30	
<212> TYPE: DNA	
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<400> SEQUENCE: 190

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<210> SEQ ID NO 191

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<212> TYPE: DNA

<213> ORGANISM: Human papillomavirus type 52

<400> SEQUENCE: 191

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<210> SEQ ID NO 192

<211> LENGTH: 30

<212> TYPE: DNA

<213> ORGANISM: Human papillomavirus type 52

<400> SEQUENCE: 192

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<211> LENGTH: 30

<212> TYPE: DNA

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<400> SEQUENCE: 193

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<210> SEQ ID NO 194

<211> LENGTH: 30

<212> TYPE: DNA

<213> ORGANISM: Human papillomavirus type 52

<400> SEQUENCE: 194

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<210> SEQ ID NO 195

<211> LENGTH: 30

<212> TYPE: DNA

<213> ORGANISM: Human papillomavirus type 52

<400> SEQUENCE: 195

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<210> SEQ ID NO 196

<211> LENGTH: 30

<212> TYPE: DNA

<213> ORGANISM: Human papillomavirus type 52

<400> SEQUENCE: 196

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<210> SEQ ID NO 197

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<212> TYPE: DNA

<213> ORGANISM: Human papillomavirus type 53

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<212> TYPE: DNA

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<213> ORGANISM: Human papillomavirus type 53

<400> SEQUENCE: 198

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<210> SEQ ID NO 199

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<212> TYPE: DNA

<213> ORGANISM: Human papillomavirus type 53

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<210> SEQ ID NO 201

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<212> TYPE: DNA

<213> ORGANISM: Human papillomavirus type 56

<400> SEQUENCE: 201

gtactgctac agaacagtttta agtaaatatgg 30

<210> SEQ ID NO 202

<211> LENGTH: 30

<212> TYPE: DNA

<213> ORGANISM: Human papillomavirus type 56

<400> SEQUENCE: 202

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<210> SEQ ID NO 203

<211> LENGTH: 30

<212> TYPE: DNA

<213> ORGANISM: Human papillomavirus type 58

<400> SEQUENCE: 203

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<210> SEQ ID NO 204

<211> LENGTH: 30

<212> TYPE: DNA

<213> ORGANISM: Human papillomavirus type 58

<400> SEQUENCE: 204

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<210> SEQ ID NO 205

<211> LENGTH: 30

<212> TYPE: DNA

<213> ORGANISM: Human papillomavirus type 58

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<210> SEQ ID NO 206

<211> LENGTH: 30

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<212> TYPE: DNA
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<210> SEQ ID NO 207
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<212> TYPE: DNA
<213> ORGANISM: Human papillomavirus type 61

<400> SEQUENCE: 207
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<210> SEQ ID NO 208
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<400> SEQUENCE: 208
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<210> SEQ ID NO 209
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<212> TYPE: DNA
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<400> SEQUENCE: 209
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<210> SEQ ID NO 210
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<400> SEQUENCE: 210
agctaaaagg acattaacta aatatgatgc 30

<210> SEQ ID NO 211
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<213> ORGANISM: Human papillomavirus type 66

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<210> SEQ ID NO 212
<211> LENGTH: 30
<212> TYPE: DNA
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<400> SEQUENCE: 212
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<210> SEQ ID NO 213
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<212> TYPE: DNA
<213> ORGANISM: Human papillomavirus type 66

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<210> SEQ ID NO 214

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<400> SEQUENCE: 215

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<210> SEQ ID NO 216
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<212> TYPE: DNA
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<400> SEQUENCE: 216

ctcacaatcg ctttctgcta catataatag                                30

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<400> SEQUENCE: 217

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<210> SEQ ID NO 218
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<213> ORGANISM: Human papillomavirus

<400> SEQUENCE: 218

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<210> SEQ ID NO 219
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<213> ORGANISM: Human papillomavirus

<400> SEQUENCE: 219

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<210> SEQ ID NO 220
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<213> ORGANISM: Human papillomavirus

<400> SEQUENCE: 220

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<210> SEQ ID NO 221
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Human papillomavirus

<400> SEQUENCE: 221

cgctaccctcag aaggccctctg aatatgaccc                                30

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<210> SEQ ID NO 222	
<211> LENGTH: 30	
<212> TYPE: DNA	
<213> ORGANISM: Human papillomavirus	
<400> SEQUENCE: 222	
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<210> SEQ ID NO 223	
<211> LENGTH: 27	
<212> TYPE: DNA	
<213> ORGANISM: Human papillomavirus	
<400> SEQUENCE: 223	
cacaaagttt aaggaatatt taaggca	27
<210> SEQ ID NO 224	
<211> LENGTH: 30	
<212> TYPE: DNA	
<213> ORGANISM: Human papillomavirus	
<400> SEQUENCE: 224	
aacaaccact gaatatgacc ccacaaagtt	30
<210> SEQ ID NO 225	
<211> LENGTH: 30	
<212> TYPE: DNA	
<213> ORGANISM: Human papillomavirus	
<400> SEQUENCE: 225	
ccgttagtacc aaccttacca tttgtgctgc	30
<210> SEQ ID NO 226	
<211> LENGTH: 30	
<212> TYPE: DNA	
<213> ORGANISM: Human papillomavirus	
<400> SEQUENCE: 226	
catttgtct gttcccagt ctggcacaga	30
<210> SEQ ID NO 227	
<211> LENGTH: 30	
<212> TYPE: DNA	
<213> ORGANISM: Human papillomavirus	
<400> SEQUENCE: 227	
cacacaaaaca ccctctgaca catacaaggc	30
<210> SEQ ID NO 228	
<211> LENGTH: 30	
<212> TYPE: DNA	
<213> ORGANISM: Human papillomavirus	
<400> SEQUENCE: 228	
ctacctacta catatgacaa cacaaagttc	30
<210> SEQ ID NO 229	
<211> LENGTH: 30	
<212> TYPE: DNA	
<213> ORGANISM: Human papillomavirus	
<400> SEQUENCE: 229	
atccactgag tctgtgctac ctactacata	30

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<210> SEQ ID NO 230
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Human papillomavirus

<400> SEQUENCE: 230

tttaacactg tgtgcttcta cacaaaatgg 30

<210> SEQ ID NO 231
<211> LENGTH: 138
<212> TYPE: DNA
<213> ORGANISM: Human papillomavirus type 6

<400> SEQUENCE: 231

tttgttactg tggttagatac cacacgcagt accaacatga cattatgtgc atccgttaact	60
acatcttcca catacaccaa ttctgattat aaagagtaca tgcgtcatgt ggaagagtat	120
gatttacaat ttatttt	138

<210> SEQ ID NO 232
<211> LENGTH: 136
<212> TYPE: DNA
<213> ORGANISM: Human papillomavirus type 11

<400> SEQUENCE: 232

tttgttactg tggttagatac cacacgcagt acaaatatga cactatgtgc atctgtctaa	60
atctgttaca tacactaatt cagattataa ggaatacatgt cgccatgtgg aggagttga	120
tttacagttt attttt	136

<210> SEQ ID NO 233
<211> LENGTH: 144
<212> TYPE: DNA
<213> ORGANISM: Human papillomavirus type 13

<400> SEQUENCE: 233

tttgttactg tagttgatac tacacgcagt actaacatga ctgtgtgtgc agccactaca	60
tcatctcttt cagacacata taaggccaca gaatataaac agtacatgcg acatgttagaa	120
gaatttgatt tacaatttat tttt	144

<210> SEQ ID NO 234
<211> LENGTH: 141
<212> TYPE: DNA
<213> ORGANISM: Human papillomavirus type 16

<400> SEQUENCE: 234

tttgttactg tggtgatac tacacgcagt acaaatatgt cattatgtgc tgccatatct	60
acttcagaaa ctacatataa aaataactaac tttaaggagt acctacgaca tggggaggaa	120
tatgatttac agttttttt t	141

<210> SEQ ID NO 235
<211> LENGTH: 144
<212> TYPE: DNA
<213> ORGANISM: Human papillomavirus type 18

<400> SEQUENCE: 235

tttgttactg tggttagatac cactcccagt accaatttaa caaatgtgc ttctacacag	60
tccctgtac ctggcaata tgatgctacc aaatthaagc agtatacgac acatgttgag	120
gaatatgatt tgcagtttat tttt	144

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<210> SEQ ID NO 236
<211> LENGTH: 144
<212> TYPE: DNA
<213> ORGANISM: Human papillomavirus type 26

<400> SEQUENCE: 236

tttgttacct gtgttgatac cacccgcagt actaacctta ccattagtagc attatctgca	60
gcatctgcat ccactccatt taaaccatct gattataaac aatttataag acatggcgaa	120
gaatatgaat tacaatttat attt	144

<210> SEQ ID NO 237
<211> LENGTH: 137
<212> TYPE: DNA
<213> ORGANISM: Human papillomavirus type 30

<400> SEQUENCE: 237

tttgttactg tgtggacacc actaggaaca caaacatgac tatatctgca accacacaaa	60
cgttatccac atataattca agccaaatta aacagtatgt aagacatgta gaggaatatg	120
aattacagtt tgtgttt	137

<210> SEQ ID NO 238
<211> LENGTH: 141
<212> TYPE: DNA
<213> ORGANISM: Human papillomavirus type 31

<400> SEQUENCE: 238

tttgttactg tggttagatac cacacgtagt accaatatgt ctgtttgtgc tgcaattgca	60
aacagtgtata ctagattaa aagtagtaat tttaaagagt atttaaagaca tggtgaggaa	120
tttgatttac aatttatatt t	141

<210> SEQ ID NO 239
<211> LENGTH: 138
<212> TYPE: DNA
<213> ORGANISM: Human papillomavirus type 32

<400> SEQUENCE: 239

tttctaactg ttgtggatac tacccgtagt actaacatgac ctgtgtgtgc tactgttaaca	60
actgaagaca catacaagtc tactaacttt aaggaatatc tacgccccatgc agaggaatat	120
gatatacagt ttatattt	138

<210> SEQ ID NO 240
<211> LENGTH: 138
<212> TYPE: DNA
<213> ORGANISM: Human papillomavirus type 33

<400> SEQUENCE: 240

tttgttactg tggttagatac cactcgcaagt actaatatga ctttatgcac acaagtaact	60
agtgcacatgtatataaaaaa tgaaaatttt aaagaatata taagacatgt tgaagaatat	120
gatctacagt ttgttttt	138

<210> SEQ ID NO 241
<211> LENGTH: 140
<212> TYPE: DNA
<213> ORGANISM: Human papillomavirus type 35

<400> SEQUENCE: 241

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tttgttactg tagtgatac aaccgttagt acaaatatgt ctgtgtgtc tgctgtgtct	60
tctagtcaca gtacatataa aaatgacaat tttaaggaat attttaggcat ggtgaagaat	120
atgatttaca gtttattttt	140
<210> SEQ ID NO 242	
<211> LENGTH: 144	
<212> TYPE: DNA	
<213> ORGANISM: Human papillomavirus type 39	
<400> SEQUENCE: 242	
tttcttactg ttgtggacac taccgttagt accaacttta cattatctac ctctatagag	60
tcttccatac cttctacata tgatccttct aagtttaagg aatataccag gcacgtggag	120
gagttatgatt tacaattttt attt	144
<210> SEQ ID NO 243	
<211> LENGTH: 145	
<212> TYPE: DNA	
<213> ORGANISM: Human papillomavirus type 40	
<400> SEQUENCE: 243	
tttgttacag ttgttagacac cactcgtagc actaatttaa ctttatgtgc tgccacacag	60
tccccccacac caaccccaata taataacagt aatttcaagg aatatttgcg tcatggggag	120
gagtttgatt tgcagttta tttttt	145
<210> SEQ ID NO 244	
<211> LENGTH: 138	
<212> TYPE: DNA	
<213> ORGANISM: Human papillomavirus type 42	
<400> SEQUENCE: 244	
tttttaactg tgggtgatac taccgtact actaacatga ctttgtgtgc cactgcaaca	60
tctgggtgata catatacagc tgctaatttt aaggaatatt taagacatgc tgaagaatat	120
gatgtgcaat ttatattt	138
<210> SEQ ID NO 245	
<211> LENGTH: 143	
<212> TYPE: DNA	
<213> ORGANISM: Human papillomavirus type 43	
<400> SEQUENCE: 245	
tttgttacag ttgttagatac cactcgtagt acaaacttaa cgttatgtgc ctctactgac	60
cctactgtgc ccagtagatac tgacaatgca aagtttaagg aataacctgag gcatgtggag	120
aatatgtatct gcagttataa ttt	143
<210> SEQ ID NO 246	
<211> LENGTH: 144	
<212> TYPE: DNA	
<213> ORGANISM: Human papillomavirus type 44	
<400> SEQUENCE: 246	
tttgttactg ttgttagatac taccgttagt acaaacatga caaatatgtgc tgccactaca	60
cactccctc cgtctacata tactagtgaa caatataagg aatacatgag acatgttgag	120
gagtttgact tacaattttt gttt	144
<210> SEQ ID NO 247	
<211> LENGTH: 144	

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<212> TYPE: DNA
<213> ORGANISM: Human papillomavirus type 45

<400> SEQUENCE: 247

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aatcctgtgc caagtcata tgaccctact aagtttaagg agtatagtac acatgtggag	120
gaatatgatt tacagtttat tttt	144

<210> SEQ ID NO 248
<211> LENGTH: 141
<212> TYPE: DNA
<213> ORGANISM: Human papillomavirus type 51

<400> SEQUENCE: 248

tttattacct gtcttgatac taccagaagt acaaatttaa ctattagcac tgccactgct	60
ggggtttccc caacatttaa tccaagtaac tttaagcaat atattaggca tggggaaagag	120
tatgaattgc aattttttt t	141

<210> SEQ ID NO 249
<211> LENGTH: 138
<212> TYPE: DNA
<213> ORGANISM: Human papillomavirus type 52

<400> SEQUENCE: 249

tttgtcacag ttgtggatac cactcgtagc actaacatga ctttatgtgc tgaggtaaa	60
aaggaaagca catataaaaa tgaaaatttt aaggaatacc ttctgtatgg cgaggaattt	120
gatttacaat ttatTTT	138

<210> SEQ ID NO 250
<211> LENGTH: 138
<212> TYPE: DNA
<213> ORGANISM: Human papillomavirus type 53

<400> SEQUENCE: 250

tttgtaactg ttgtggatac caccaggaat acaaacatga ctcttccgc aaccacacag	60
tctatgtcta catataattc aaagcaaattt aaacagtatg ttagacatgc agaggaatatt	120
gaatttacaat ttgtgttt	138

<210> SEQ ID NO 251
<211> LENGTH: 137
<212> TYPE: DNA
<213> ORGANISM: Human papillomavirus type 54

<400> SEQUENCE: 251

tttttaacag ttgttagatac cacccgttagt actaacctaa cattgtgtgc tacagcatcc	60
acgcaggata gcttaataa ttctgacttt agggagtata ttagacatgt taggaatatg	120
atttacagtt tatattt	137

<210> SEQ ID NO 252
<211> LENGTH: 138
<212> TYPE: DNA
<213> ORGANISM: Human papillomavirus type 56

<400> SEQUENCE: 252

tttgttactg tagtagatac tactagaagt actaacatga ctattagtagc tgctacagaa	60
cagttaagta aatatgtgc acgaaaaattt aatcagtagcc ttagacatgt ggaggaatatt	120

gaattacaat ttgtttt	138
<210> SEQ ID NO 253	
<211> LENGTH: 138	
<212> TYPE: DNA	
<213> ORGANISM: Human papillomavirus type 58	
<400> SEQUENCE: 253	
tttgttaccc tggtgatac cactcgtagc actaatatga cattatgcac tgaagtaact	60
aaggaaggta catataaaaaa tgataatttt aaggaatatg tacgtcatgt tgaagaatat	120
gacttacagt ttgtttt	138
<210> SEQ ID NO 254	
<211> LENGTH: 144	
<212> TYPE: DNA	
<213> ORGANISM: Human papillomavirus type 59	
<400> SEQUENCE: 254	
tttttaacag ttgtgatac tactcgac accaatcttt ctgtgtgtgc ttctactact	60
tcttcatttc ctaatgtata cacacctacc agttttaaag aatatgccag acatgtggag	120
gaatttgatt tgcagtttat attt	144
<210> SEQ ID NO 255	
<211> LENGTH: 141	
<212> TYPE: DNA	
<213> ORGANISM: Human papillomavirus type 61	
<400> SEQUENCE: 255	
tttgtaaccg ttgtggatac cacccgcagt actaatttaa ccatttgcac tgctacatcc	60
ccccctgtat ctgaatataa agccacaago tttagggaaat atttgcgcac tacagaggag	120
tttgatttgc aatttatttt t	141
<210> SEQ ID NO 256	
<211> LENGTH: 137	
<212> TYPE: DNA	
<213> ORGANISM: Human papillomavirus type 62	
<400> SEQUENCE: 256	
tttgttactg ttgtggatac taccagaagt actaattttt ctattttgc cgcctccact	60
gctgcagcag aatacacccgc taccacactt agggattttt gcgacacacg gaggaatttgc	120
atttgcaatt tatattt	137
<210> SEQ ID NO 257	
<211> LENGTH: 138	
<212> TYPE: DNA	
<213> ORGANISM: Human papillomavirus type 66	
<400> SEQUENCE: 257	
tttgttactg ttgtggatac taccagaagc accaacatga ctattaatgc agctaaaagc	60
acatcaaacta aatatgtatgc ccgtgaaatc aatcaataacc ttgcgcatgt ggaggaatatttgc	120
gaactacagt ttgtgttt	138
<210> SEQ ID NO 258	
<211> LENGTH: 138	
<212> TYPE: DNA	
<213> ORGANISM: Human papillomavirus type 67	
<400> SEQUENCE: 258	

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tttgttactg ttttagaca ctacacgtag taccaacatg acttatgtt ctgaggaaaa	60
atcagaggct acatacaaaa atgaaacttt aaggaatacc ttagacatgt ggaagaatat	120
gatttgcagt ttatattt	138
<210> SEQ ID NO 259	
<211> LENGTH: 144	
<212> TYPE: DNA	
<213> ORGANISM: Human papillomavirus type 68	
<400> SEQUENCE: 259	
tttcttacgg ttgtggatac aacgcgcagt actaattta cattgtccac tactacagac	60
tctactgtac cagctgtgta tgattctaat aaatttaagg aatatgttag gcatgttgag	120
gaatatgatt tgcagtttat attt	144
<210> SEQ ID NO 260	
<211> LENGTH: 144	
<212> TYPE: DNA	
<213> ORGANISM: Human papillomavirus type 69	
<400> SEQUENCE: 260	
tttgttactt gtgttagatac taccgcgcagt accaacctca ctattagtagc tgtatctgca	60
caatctgcac ctgccacttt taaaccatca gattataagg agtttataag gcatggtag	120
gaatatgaat tacagtttat attt	144
<210> SEQ ID NO 261	
<211> LENGTH: 144	
<212> TYPE: DNA	
<213> ORGANISM: Human papillomavirus type 70	
<400> SEQUENCE: 261	
tttattactg tggggacac tacacgtgt actaattttt cattgtctgc ctgcaccgaa	60
acggccatac ctgctgtata tagccctaca aagtttaagg aatatactag gcatgtggag	120
gaatatgatt tacaattttt attt	144
<210> SEQ ID NO 262	
<211> LENGTH: 137	
<212> TYPE: DNA	
<213> ORGANISM: Human papillomavirus type 71	
<400> SEQUENCE: 262	
tttgtaacag ttgtgacaca tcacgtgtt caaatatgtc catctgtgtt accaaaactg	60
ttgagtctac atataaagcc tctagttca tggaatattt gagacatgga gaagaatttgc	120
atttgcattt tatattt	137
<210> SEQ ID NO 263	
<211> LENGTH: 141	
<212> TYPE: DNA	
<213> ORGANISM: Human papillomavirus type 72	
<400> SEQUENCE: 263	
tttgtgacag ttgttagatac tactcgacgt actaatgtaa ctatttgcgtt tgccacagcg	60
tcctctgtat cagaatatac agcttctaat tttcgatgtt atcttcgcacactgaggaa	120
tttgatttgc agtttatattt t	141
<210> SEQ ID NO 264	

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<211> LENGTH: 147
<212> TYPE: DNA
<213> ORGANISM: Human papillomavirus type 73

<400> SEQUENCE: 264

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gcttagtagct ctactacaac gtagccaaac tctaattttt aggaatattt aagacatgca      120
gaagagttt atttacagtt tgttttt                                         147

<210> SEQ ID NO 265
<211> LENGTH: 144
<212> TYPE: DNA
<213> ORGANISM: Human papillomavirus type 74

<400> SEQUENCE: 265

tttgttacag ttgtggatac cacacgcagt actaacatga ctgtgtgtgc tcctacctca      60
caatcgccctt ctgctacata taatagttca gactacaaac aatacatgct acatgtggag      120
gaattttgatt tgcaattttt tttt                                         144

<210> SEQ ID NO 266
<211> LENGTH: 138
<212> TYPE: DNA
<213> ORGANISM: Human papillomavirus type 81

<400> SEQUENCE: 266

tttgttacag tggggatac taccagaagc accaattttt ctatggcac agctacatct      60
gctgctgcag aatacaaggc ctctaacttt aaggaatttc tgccatcac agaggaatat      120
gatttgcagt ttatttt                                         138

<210> SEQ ID NO 267
<211> LENGTH: 144
<212> TYPE: DNA
<213> ORGANISM: Human papillomavirus type 82

<400> SEQUENCE: 267

tttattactt gtgttgacac tactaaaagt accaattttt ccattagcac tgctgttact      60
ccatctgttg cacaacatt tactccagca aactttaagc agtacattag gcatggggaa      120
gaatatataat tgcaattttt attt                                         144

<210> SEQ ID NO 268
<211> LENGTH: 138
<212> TYPE: DNA
<213> ORGANISM: Human papillomavirus type 83

<400> SEQUENCE: 268

tttgttacag ttgttagatac taccgcagt accaatattt ctatccagc tgctgctaca      60
caggctaatg aatacacagc ctctaacttt aaggaatacc tccgcacac agaggaatat      120
gacttacagg ttatattt                                         138

<210> SEQ ID NO 269
<211> LENGTH: 138
<212> TYPE: DNA
<213> ORGANISM: Human papillomavirus type 84

<400> SEQUENCE: 269

tttgtcacgg ttgttagatac cacccgcagc accaattttt ctattatgc tgctaccaac      60
accgaatcag aatataaacc taccaatttt aaggaatacc taagacatgt ggaggaatat      120

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gatttgcagt ttatattc	138
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<210> SEQ ID NO 270	
<211> LENGTH: 144	
<212> TYPE: DNA	
<213> ORGANISM: Human papillomavirus type 85	
<400> SEQUENCE: 270	
tttataactg tggtagacac aacacgtagt accaatctta ccttatctac tgcaactact	60
aatccagttc catctatata tgaaccttct aaatthaagg aatacacacg ccatgttagag	120
gaatatgatt tacaattttt attt	144

<210> SEQ ID NO 271	
<211> LENGTH: 138	
<212> TYPE: DNA	
<213> ORGANISM: Human papillomavirus type 86	
<400> SEQUENCE: 271	
tttgttactg tggcgacac cacccgcagt actaatttta ctattagtgcc cgctaccagg	60
aaggccctcg aatatgaccc ctctaagttt aatgaatatc taaggcatgc agaggaatat	120
gatttgcata ttatTTTT	138

<210> SEQ ID NO 272	
<211> LENGTH: 138	
<212> TYPE: DNA	
<213> ORGANISM: Human papillomavirus type 87	
<400> SEQUENCE: 272	
tttgtaacgg ttgttatac tactcgagt accaatttta ctattagtgcc tgccactcaa	60
acaaccactg aatatgaccc cacaaggTTT aaggaatatt taaggcatgt ggaggaatat	120
gatttacagt ttatTTTT	138

<210> SEQ ID NO 273	
<211> LENGTH: 139	
<212> TYPE: DNA	
<213> ORGANISM: Human papillomavirus type 89	
<400> SEQUENCE: 273	
tttgttactg tggggatac cacccgtagt accaaccctta ccatttgcc tgcttccagg	60
tctggcacag aaatacagtt ctacacgctt taaggaatat ttaagacaca ctgaggaata	120
tgacctacag ttatTTTC	139

<210> SEQ ID NO 274	
<211> LENGTH: 138	
<212> TYPE: DNA	
<213> ORGANISM: Human papillomavirus type 90	
<400> SEQUENCE: 274	
tttgtaactg tgggtatac tacacgtac accaatatga ctatttgcc cacacaaaca	60
ccctctgaca catacaaggc ttccaatttt aaagagtaca tgcgcattgg cgaggaattt	120
gatttgcagt ttatTTTC	138

<210> SEQ ID NO 275	
<211> LENGTH: 144	
<212> TYPE: DNA	
<213> ORGANISM: Human papillomavirus type 91	

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<400> SEQUENCE: 275

tttgttaactg ttgtggatac aactcgacg actaacttaa ccttgtgtgc atccactgag	60
tctgtgtac ctactacata tgacaacaca aagttcaaag aatattaag gcatgcagaa	120
gaattttagt tacagtttat attt	144

<210> SEQ ID NO 276

<211> LENGTH: 143

<212> TYPE: DNA

<213> ORGANISM: Human papillomavirus type 97

<400> SEQUENCE: 276

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aatggcgtag ctaccacata tgaccctaca aatataaaca gtatagtaga catgtggaa	120
agtatgattt acagtttatt ttt	143

The invention claimed is:

1. A method of detecting and typing a human papilloma-virus (HPV) type infection in a sample comprising:

- a) providing a sample suspected of comprising at least one HPV type;
- b) adding to the sample primers suitable for amplifying the L1 region of HPV;
- c) incubating the sample under conditions suitable for DNA amplification;
- d) adding a probe consisting of the nucleotide sequence of SEQ ID NO:46, said probe binding to only HPV type 97 under hybridization conditions, said probe further comprising a unique tag, said unique tag comprising a combination of two fluorescent dyes;

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e) incubating said probe and said sample under conditions suitable for hybridization; and
f) detecting hybridization of said probe.

2. The method according to claim 1 wherein the unique tag is a combination of different ratios of red and infra-red fluorophores.

3. The method according to claim 1 wherein the primers comprise GP5+/GP6+.

4. The method according to claim 1 wherein the primers comprise GP5+/GP6+ and MY09/MY11.

5. The method according to claim 1 wherein at least one of the primers is exonuclease resistant.

6. The method according to claim 5 wherein exonuclease is added prior to step (d).

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